Bumblebee foraging patterns: plant-pollinator network dynamics and robustness

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Bumblebee foraging patterns: plant-pollinator network dynamics and robustness

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I am the author of this thesis, and the work described therein was carried out by myself personally, with the exception of Chapter 2, a published paper in which I collaborated with Dr Michael Pocock and Dr Richard James. All three authors designed the methodology of the paper, discussed the results and commented on the manuscript at all stages. I coded the models and analysed the data with technical advice and support from Dr Richard James and Michael Pocock. I drafted the manuscript. All authors contributed critically to the drafts and gave final approval for publication of the paper.

Candidate's signature:

Contents

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Abstract

Pollination is a vital ecosystem service that supports and maintains ecosystems globally. Additionally, pollination has high economic value to humans and is essential for our global food production requirements. Recent threats to pollination services and declines in insect pollinators have galvanised a large and varied body of research with the aim of protecting pollination services. One approach, adopted in this thesis, is to examine plant-pollinator communities using network theory; enabling us to visualise, quantify and analyse communities as a whole. Network theory is increasingly used by ecologists; the study of ecological systems is ideal for a network approach. However, we identify a gap in our understanding and consideration of community dynamics when it comes to using plant-pollinator network data. In this thesis, our overarching goal is to better understand the dynamics of plant-pollinator communities over a range of time scales. We combine theoretical, observational and experimental techniques to examine how plant-pollinator networks change over time in terms of network structure and pollinator behaviour. We first examine the robustness of plant-pollinator networks to theoretical extinctions over evolutionary time. We then determine how much a plant-pollinator community changes over a pollination season, and over two years, through observations of flowering phenologies. Thirdly, we determine the impacts on network structure and pollinator behaviour of the experimental removal of a plant species from a plant-pollinator community, also focussing on bumblebee species to explore species level responses. Finally, we focus on the foraging patterns of bumblebee individuals to understand how foraging behaviour, on the timescales of sequential flower visits, can facilitate bumblebee species adapting to longer term changes in their foraging environment and ultimately how individual behaviour responds to and impacts the dynamics of the whole plant-pollinator community.

Chapter 1

Introduction

Pollination is a vital and economically valuable ecological process (Losey and Vaughan, 2006; Winfree, Gross and Kremen, 2011; Hanley *et al.*, 2015). Recent declines in insect pollinators threaten pollination services and the stability of ecosystems globally (Vanbergen and Initiative, 2013; Potts *et al.*, 2016). In response, recent research has focused on understanding declines in pollinators, the specific threats that they face and how we can protect pollination services for the future (Gill et al., 2016; Ollerton, 2017). Areas of focus include for example; understanding the impacts of pesticides on specific pollinator species (Goulson et al., 2015; Neumann et al., 2015; Tsvetkov et al., 2017) and using species distributions and climate models to understand how pollinators and plant species respond to climate change (Menzel *et al.*, 2006; Memmott *et al.*, 2007; Kelly and Goulden, 2008). Some studies have taken the approach of focusing in on particular species to contribute detailed information about individual pieces of the puzzle whilst others have taken a more system wide approach to improve our understanding of pollination communities as a whole.

With this thesis, we aim to contribute to the protection of pollination services through a better understanding of plant-pollinator community dynamics. We adopt a 'network theory' approach to explore plant-pollinator communities in terms of the mutualistic interactions between plant and pollinator species. We use theoretical techniques, field observations and field experiments to examine how interactions are impacted by temporal changes in the community (or network). In addition, we explore how species play a role in creating, as well as responding to, community dynamics. To do this we focus on the temporal dynamics and foraging behaviour of bumblebee species. Bumblebees are an ideal focal species because they are relatively well studied (compared to some other insect pollinators) and are easy to work with in the wild due to their size, docility and foraging behaviour. In addition, declines have been recorded in many bumblebee species and so a greater understanding of their role in plant-pollinator communities is valuable for conservation (Carvell *et al.*, 2006).

Overall, this thesis is made up of two parts. The first part (Chapter 2) takes an entirely theoretical approach to understanding the stability of plant-pollinator communities. A range of theoretical approaches have greatly advanced our understanding of plant-pollinator communities. We focus on 'knockout' extinction models of 'robustness' which quantify community robustness to extinctions over ecological time (abstract timescales over many seasons). These models make use of plant-pollinator network data that has been collected in the field over much shorter timescales (days, seasons and sometimes years). Plant-pollinator networks are often assumed to be representative and static for the purposes of modelling. In the second part of this thesis (Chapters 3, 4 and 5) we will challenge these

assumptions and explore how plant-pollinator communities are dynamic over a range of timescales. In Chapter 3 we present an analysis of plant-pollinator community dynamics in terms of the turnover of flowering plant species over a pollination season (March-September) and the corresponding turnover of bumblebee foraging interactions. In Chapter 4 we present an experimental manipulation of a plant-pollinator network and in Chapter 5 we focus on the foraging dynamics of bumblebee species.

In the first half of this introductory chapter, we introduce the main concepts covered in this thesis and provide an overview of existing research into plant-pollinator networks. In sections 1.1 to 1.3 we give a short summary of pollination and its ecological importance followed by an overview of insect pollinators and bees in particular. In section 1.4, we focus in on bumblebees as pollinators, giving a summary of bumblebee ecology, why bumblebees are important pollinators and why we focus on them, out of all the insect pollinators, in this thesis. We then zoom back out again to give an overview of current declines in insect pollinators (1.5) and discussion some of the main recognised threats to pollination services (1.6). In the second half of this chapter, we introduce network theory and the value in a network approach to understanding plant-pollinator communities. We start with a brief introduction to network theory in section 1.6, discussing the key concepts and range of applications. We then bring the focus in on plant-pollinator networks, reasoning why a networks approach is valuable for understanding plant-pollinator communities and how this approach has been used in recent years in section 1.7. Finally, in section 1.8 we summarise the main motivations of this thesis and set out the key objectives of each chapter in section 1.9.

1.1 A brief overview of pollination

Pollination is the transfer of pollen grains from the anthers (male reproductive organ) to the stigma (female reproductive organ) of a plant (Hine & Martin, 2015), (see Figure 1.1). Successful pollination leads to fertilization and germination, ultimately allowing a plant to reproduce (Waser & Forrest, 2014). Pollination can occur between the anthers and stigma of the same plant, known as self-pollination, or between plants of the same species, known as cross pollination. Many plant species are capable of reproduction through both self and cross fertilisation, though still require some degree of cross-fertilization in order to maintain genetic diversity and a healthy seed set (Kearns, Inouye and Waser, 1998; Ollerton, Winfree and Tarrant, 2011).

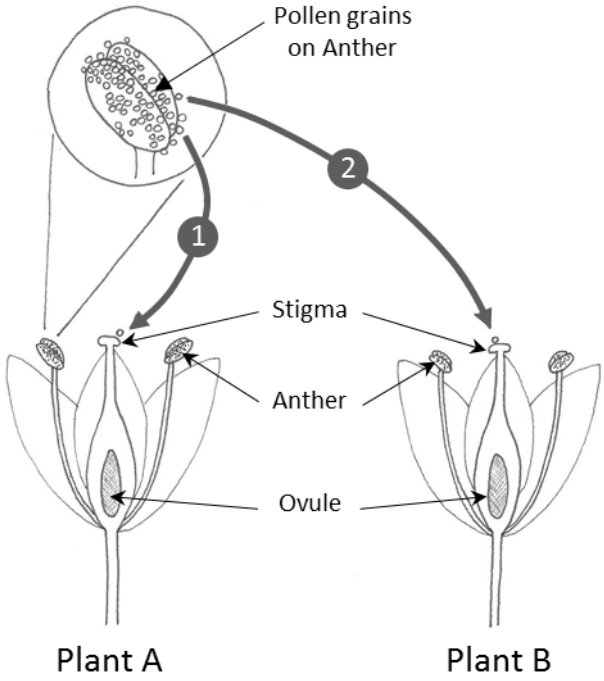

Figure 1.1 The pollination of an angiosperm. For pollination to occur, a grain of pollen (not to scale) must be transferred from the anthers (male reproductive organ) to the stigma (female reproductive organ). Successful pollination leads to the fertilization of the ovule (egg). Self-pollination occurs when pollen is transferred from the anthers to the stigma of the same plant, as indicated by arrow 1. Cross pollination occurs when the pollen is transferred to the sigma of a different plant (of the same species), as indicated by arrow 2.

Pollination is a vital ecological process. Plants convert energy from the sun into a form that can then be used by other living organisms; ecological systems largely rely on plant species as their basal form of energy. In human terms, pollination is considered an ecosystem service; a benefit that humans gain from ecosystem functions which has an economic value (Mace, 2008). Approximately 1500 crop species worldwide are dependent on pollination (by animals) and an estimated 35% of global food production in terms of volume is reliant on animal mediated pollination (Klein *et al.*, 2007). Overall, pollination is vital to maintain functioning ecosystems and humans hugely benefit from pollination in terms of food, ecosystem stability and the ascetic and cultural value of diverse ecosystems (Gill *et al.*, 2016)

1.2 Pollinators

In the case of cross pollination (and sometimes self-pollination), an external 'agent' is required to facilitate the transfer of the pollen grains. A minority of angiosperms are pollinated by abiotic agents such as wind (Culley, Weller and Sakai, 2002), whilst the majority are pollinated by animals. An animal 'agent' is referred to as a 'pollinator'. Estimates agree that the proportion of animal pollinated angiosperms is high. For example, a commonly cited review of published and unpublished data estimated that of the (approximate) 352,000 angiosperm species worldwide, 308,000 (= 88%) are pollinated by animals (Ollerton, Winfree & Tarrant, 2011). Without animal mediated pollination, approximately 75% of globally important crops including most fruits, seeds and nuts as well as high-value crops such as coffee, cocoa and oilseed rape would be negatively impacted in terms of yield (Gallai et al., 2009).

Most animal mediated pollination is carried out by insects, particularly in temperate regions (Hanley *et al.*, 2015) thought other animal pollinators include some species of birds, lizards, bats, monkeys and rodents (Potts *et al.*, 2016). The major insect pollinators are those of the orders Hymenoptera (ants, bees, wasps, and sawflies), Lepidoptera (butterflies and moths), Diptera (the true flies) and Coleoptera (beetles and weevils), (Waser, 2007). The Hymenoptera, specifically bees, are considered to be particularly important as they are the primary pollinators for most plants that require insect pollination (Free, 1979; Klein *et al.*, 2007). Bees are known to visit more than 90% of the top 107 global crop types (Potts *et al.*, 2016). Bees not only make up a large proportion of known pollinators but also are particularly efficient pollinators due to aspects of their physiology and behaviour. Approximately 84% of crop species produced in Europe rely on insect pollination and bees in particular (Williams, 1994). Native bees contribute substantially to the pollination of major crops such as coffee, watermelon, tomato, blueberry and rapeseed (oil) (Winfree, Gross and Kremen, 2011). Insect pollinators are a vital part the ecosystem service of pollination and bees in particular play an important role in maintaining the diversity of wild plants and food production.

1.3 Bees as pollinators

Bees are frequent visitors to flowering plants because they require the nectar and pollen produced by the flower to survive. Nectar is a source of sugar and pollen is a source of protein and lipids; these are the main floral resources required by bees as food (Falk, 2015). When a bee visits a flowering plant to collect food resources, pollen grains become stuck to

the bee's body. When the bee visits subsequent flowers to collect more resources, the pollen is transferred between flowers, potentially resulting in cross-pollination. The bee benefits from the floral resources it collects, whilst the plant benefits from the potential bee-mediated pollination. This is a mutualistic relationship because both organisms benefit from it (Bartomeus *et al.*, 2016). This mutualism is believed to have evolved 99.6 to 65.5 million years ago (Proctor, Yeo and Lack, 1997, though see also Ren *et al.*, 2009 for possible evidence of earlier pollination) when bees evolved from hunting wasps and switched from feeding carnivorously to feeding on nectar and pollen. Over evolutionary time, bees and angiosperms have co-evolved, resulting in adaptations in both groups that strengthen their mutualistic relationship (Bawa, 1990). However, in some cases plants attract pollinators but do not provide a nectar or pollen resource for the pollinator (Renner, 2006). These interactions are therefore not mutualistic. This is very common in orchids (Jersáková, Johnson and Kindlmann, 2006). However, this thesis focuses on mutualistic pollination.

Several aspects of the general physiology of bees make them particularly effective pollinators. All bees have minute, branched hairs that pollen grains can stick to (Falk, 2015). Because these minute hairs, bees are able to carry more pollen on their bodies as they fly from flower to flower, increasing the chances of cross-pollination (see Figure 1.2). In addition, over 50 genera of bees including bumblebees, are capable of buzz pollination (Rosi-Denadai *et al.*, 2018). Also known as floral sonification, this is the process by which pollen is released from the anthers of a plant by vibrations produced by a bee (King and Buchmann, 2003). Buzz pollination has been found to be the method of pollen release in over 20,000 plant species from 70 plant families (De Luca and Vallejo-Marín, 2013). Overall, bees can be very effective pollinators, due to their specific adaptations and mutualistic relationship with plants.

In this thesis, we examine whole communities of pollinators which include many bee species among other insect pollinators (mainly Diptera and Lepidoptera). In addition to the whole community view we focus on bumblebees, specifically in terms of their foraging behaviour. In the next section (1.4) we introduce the relevant ecology of bumblebees, and in section 1.5 we discuss the threats that face bumblebees and other pollinators.

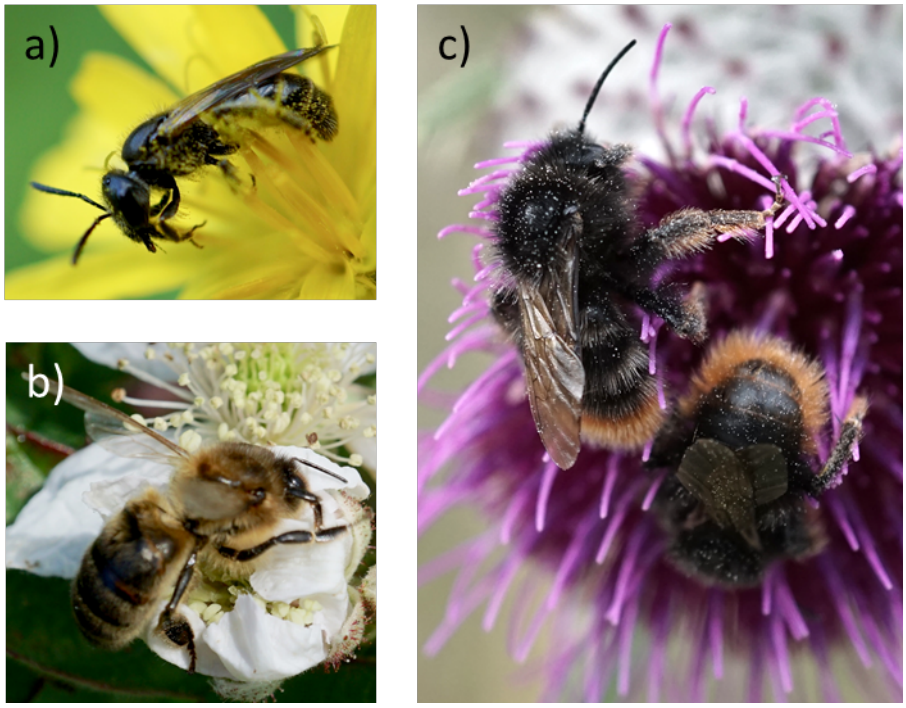

Figure 1.2 The hairy bodies of a range of bee genera: ***a)*** *a solitary bee (likely genus Halictus),* ***b)*** *a honey bee (genus Apis) and* ***c)*** *two bumblebees (genus Bombus). In* ***a)*** *and* ***c)*** *you can clearly see the small yellow and white dots that are grains of pollen being carried on the bees' hair.*

1.4 Bumblebees

There are approximately 250 described species of bumblebee (genus *Bombus*) in the world (Falk, 2015). They are largely temperate; in the UK there are 25 species, which all share a similar ecology (see Benton, 2006 for a general overview of bumblebee ecology). Bumblebees are primitively eusocial insects; they live in colonies with a simple caste structure and division of labour (Goulson, 2010, though see Sirohi *et al.*, 2015 for alternative definition of eusocial insects). The colony is divided into three castes: the queen, workers and males. There is one queen in a colony and she is key to the colony's lifecycle (Prys-Jones & Corbet, 1986). The queen lives for approximately a year, whereas the colony only survives for a number of months; from March to September is typical for UK species (Falk, 2015). The bumblebee lifecycle plays out as follows (summarised from Benton, 2006 and Prys-Jones and Corbet, 2011), (see Figure 1.3). A young queen bumblebee will emerge from hibernation in early spring and establish a nest. Nests are typically found in disused rodent holes, under grassy tussocks, under hedgerows and in bird boxes. Once the queen has found a suitable nest site, she lays eggs that hatch into worker bumblebees (all her daughters). Throughout the spring and summer, the colony grows as new workers are born; there may be 60-400 individuals depending on species, environmental conditions and other factors.

Towards the end of the summer, the colony begins to die off. The queen stops producing workers and instead produces young queens and males. These all leave the nest to mate with young queens and males from other colonies. The colony dies in late summer or autumn. In temperate regions, where most bumblebee species are found, the colony lifecycle coincides with the major flowering seasons as most temperate plant species flower at some point during the spring or summer (as shown in Figure 1.3).

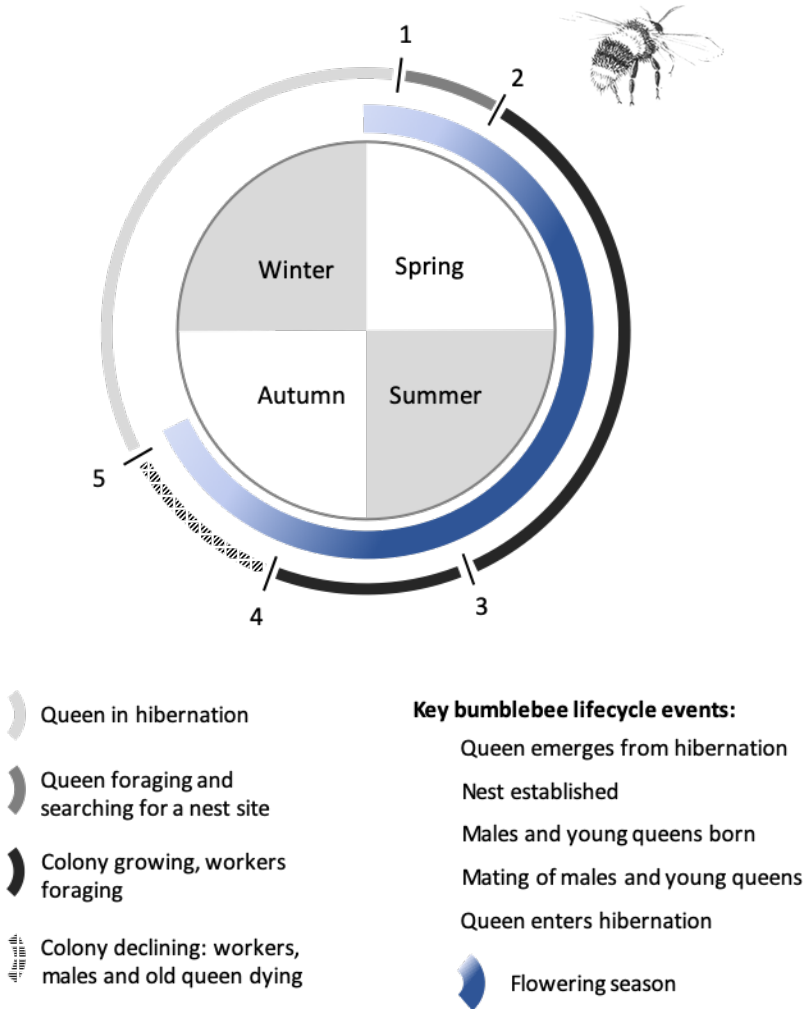

Figure 1.3 A schematic of the yearly lifecycle of a bumblebee colony (in a temperate region) with key life cycle events highlighted. Note that the period when the colony is active (from nest initiation to the mating of young queens and males, 2-4) coincides with the main flowering season, when most floral resources are available (indicated in blue). Timings of events are not exact but demonstrate the chronology of the lifecycle with approximate relative timing periods.

The exceptions to the colony lifecycle are the cuckoo bumblebees. These species, formerly classified as a separate genus (*Psithyrus*), are solitary bumblebees that are parasitic on bumblebee colonies. Cuckoo bumblebees also forage for pollen and nectar and are therefore also pollinators but are less abundant than bumblebees and probably have less impact as pollinators.

Bumblebees are social foragers; they bring food back to the nest at the end of a foraging trip to feed other workers in the nest, the queen and larvae (Benton, 2006). This means that foraging individuals need to gather enough nectar to feed themselves whilst foraging (bumblebees have one of the highest metabolisms in the animal kingdom - Goulson, 2010) as well as gathering enough resources to bring back to the nest. Excess nectar is regurgitated as honey and stored in honey pots. The honey pots could act as a buffer if the colony experiences a period of unprofitable foraging, if for example the weather conditions are unfavourable, but it is unlikely that the honey pots can support the colony for long periods as honey stores do in honeybee colonies. Therefore, it is essential that bumblebees forage often and efficiently.

Bumblebees forage for both nectar and pollen from a diverse range of flowering plants. Bumblebees have specialised mouth parts; a long tongue, known as a proboscis that unfolds and allows them to reach nectar deep in a flower and suck it up as if using a straw (Proctor, Yeo and Lack, 1996), (see Figure 1.4). This adaptation allows them to access nectar that cannot be reached by other insects with shorter proboscises (Ranta and Lundberg, 1980). It is believed that some flowering plant species have evolved alongside bumblebees to have particularly long corollas, therefore excluding other pollinators and strengthening their mutualistic relationship with bumblebees, increasing the chances of successful pollination (Inoue and Yokoyama, 2006; Suzuki, Dohzono and Hiei, 2007). As members of the family Hymenoptera, bumblebees have forked hairs which can successfully pick up pollen. Queens and workers also have pollen baskets; smooth patches on their hind tibia that are used to store pollen whilst foraging (See Figure 1.5). By storing pollen in their pollen baskets, bumblebees are able to forage on several flowers before returning to the nest with their pollen load. Like nectar, pollen is stored in the nest in pots so that is can be used to feed the colony, particularly larvae (Heinrich, 1979a). Having forked hairs and pollen baskets means bumblebees are particularly good pollinators; they carry more pollen than other insect pollinators and spend much of their time flying directly between flowers.

Figure 1.4 A Bombus hortorum worker about to feed, showing its long proboscis (indicated by the arrow) before it enters the flower.

Figure 1.5 Bumblebee pollen baskets. In the left-hand photo, the arrow (a) indicates the empty pollen basket on the hind tibia of a Bombus sylvarum worker. In the right-hand photo, the arrow (b) indicates a full pollen basket carrying a load of yellow-brown pollen on the hind tibia of a Bombus pascuorum worker.

1.5 Declines in insect pollinators

Global declines in insect pollinators are well known and widely reported (see for example Gallai *et al.*, 2009; Dicks *et al.*, 2013, 2016; Gill *et al.*, 2016). A recent study in Germany recorded a greater than 70% decline in flying insect biomass in nature protected areas over 27 years (Hallmann *et al.*, 2017). More specifically, there is evidence for considerable, recent declines in the major groups of pollinators. Swaay *et al.* reported a 50% decline in European grassland butterflies from 1990 to 2011 (van Swaay *et al.*, 2008). Honeybees have experienced recent large scale seasonal losses worldwide despite marked increases in the number of hives (Potts *et al.*, 2016). Substantial declines have been reported in species rich moth groups in the UK and Europe (Fox, 2013). In the last 50 years there have been noticeable declines in bumblebee populations. In the UK, only 8 of the 25 bumblebee species are widespread and common (Falk, 2015). Callum's bumblebee, *Bombus cullumanus;* the apple bumblebee, *Bombus pomorum;* and most recently the short-haired bumblebee, *Bombus subteraneus,* have all been declared extinct in the UK. Other species continue to decline, notably *Bombus distinguendus* (Great yellow bumblebee) and *Bombus sylvarum* (Shrill carder bee) which are currently both near to extinction in the UK. Even in groups for which data is lacking, as is the case for many Dipteran species, it is likely that declines are occurring. A recent review of the state of the worlds entomofauna (insect life) cites dramatic rates of decline which could lead to 'the extinction of 40% of the world's insect species over the next few decades' (Sánchez-Bayo and Wyckhuys, 2019). In some cases, specific factors, such as the varroa mite that preys on honeybee colonies, have been blamed for losses (Fürst *et al.* 2014). However, parallel declines across so many species suggests that there are more system wide problems that are impacting pollinator communities as a whole. In the next section (1.6) we will discuss several factors have been identified as threats to insect pollinators including modern agricultural practices, changing land use, habitat fragmentation, loss of wild flowers, and climate change.

1.6 Threats to plant-pollinator communities

Modern agricultural practices have been linked to negative impacts on insect pollinators, despite the agricultural industry being one that directly benefits from pollination. In particular, agricultural intensification, monoculture farming and the use of agrochemicals impact pollinators (Gill *et al.* 2016). Agriculture intensification in the last 5 decades has caused a major loss of biodiversity (Batary et al., 2011) with changes in agricultural policy in the 1920's linked to rapid extinctions of pollinators in the UK (Ollerton *et al.*, 2014).

Monoculture farming reduces the diversity of plants available for pollinators to feed on, as well as limiting food availability to the flowering period of the given crop. For example, oil seed rape is a rich source of nectar and pollen for bumblebees in the spring but can result in colony failure later in the year once it has been harvested and colonies are left without an alternative food source (Westphal, Steffan-Dewenter and Tscharntke, 2009). It is also possible that a single crop, particularly one that has been bred or engineered for human benefit, will not provide the full spectrum of sugars, proteins, lipids and oils required to keep certain pollinator species healthy (Bailes *et al.* 2015).

Another factor strongly associated with the agriculture industry and insect declines is the use of agrochemicals such as pesticides. Pesticides can be lethal to pollinators or can cause sub-lethal impacts on fitness (Raine *et al.,* 2015, Goulson *et al.,* 2016). Neonicotinoids are the most widely used class of insecticides in the world (Van der Sluijs *et al.* 2013). In 2018, EU member states voted on a near total ban on the use of neonicotinoids across the EU following a partial ban on three neonicotinoid chemicals in 2013. Evidence for the harmful effects of neonicotinoids on bees in particular has been under scrutiny, despite much research strongly supporting the ecological benefits of banning them (See for example Hopwood *et al.* 2012; Neumann *et al.,* 2015; Woodcock *et al.,* 2016, Tsvetkov *et al.* 2017).

Native wild flowers are a vital source of food for many insect pollinators, particularly bees (Hicks *et al.*, 2016). In the UK, there has been a 97% loss of wild flower meadows since the 1930s (Fuller, 1987) as well as observed declines in the diversity of wild flowers (Goulson *et al.,* 2015), both linked to agricultural intensification and urbanisation (Gill *et al.,* 2016). A recent study quantifying nectar sources for pollinators found evidence for substantial losses in England and Wales between the 1930s and 1970s which stabilized by 1978, and increased from 1998 to 2007 (Baude *et al.*, 2016). The removal of hedgerows as a result of agricultural intensification has resulted in a loss of both wild flowers and suitable nesting sites (Gill *et al.,* 2016). As the area of suitable habitat for insect pollinators shrinks, small islands of habitat are left in a fragmented landscape. This can lead to isolated populations of both plants and pollinators that are more vulnerable to other pressures (Xiao *et al.,* 2017). Beismeijer (2006) found evidence for parallel declines in plants and bee species in Britain and the Netherlands, which 'strongly suggest a causal connection between local extinctions of functionally linked plant and pollinator species'.

Climate change has the potential to impact pollinator species in several ways; by reducing the fitness of individuals and their ability to reproduce, by reducing suitable habitat and disrupting essential relationships between species (Memmott *et al.,* 2007). Firstly, individual insects may simply be unable to survive in a changed climate; if they are

particularly sensitive to temperature or humidity for example. More frequent extreme weather events associated with climate change may damage populations and habitats beyond recovery (Katz, 2010). Secondly, plants and pollinators in temperate regions are sensitive to the seasonality of their environments. The lifecycle events for both plants and pollinators are often dependent on seasonal cues such as temperature (Alford, 1969; Amano *et al.*, 2010). Therefore, changes in the climate may lead to the disruption of relationships between species as a result of temporal shifts in the seasons, which lead to mismatched emergence of species that rely on each other (Thackery *et al.,* 2010; Rafferty *et al.,* 2013, Ovaskainen *et al.,* 2013). Bumblebees, for example, are particularly vulnerable to both extreme weather events and seasonal shifts. The survival of the colony is dependent on the survival of the hibernating queen and if variable winters impact successful hibernation, or extreme weather in spring inhibits nest establishment, populations will quickly disappear. There is evidence to suggest that bumblebees and other pollinators are already being negatively impacted by seasonal shifts and colder spring weather through the temporal mismatch of the insect lifecycle and the flowering periods of wild flowers (for example see Menzel *et al.*, 2006; Amano *et al.*, 2010; Burkle, Marlin and Knight, 2013; Ovaskainen *et al.*, 2013).

Another widely reported effect of climate change is that species distributions shift in terms of latitude or elevation in response to changing climate conditions (Parmesan and Yohe, 2003; Hickling *et al.*, 2006; Chen *et al.*, 2011). Most insect pollinators are capable of moving in this way, helped by their ability to fly, and even plant species have been shown to shift their range (Kelly and Goulden, 2008; Lenoir *et al.*, 2008). However, not all species are equally capable of adapting in this way, causing potential mismatches in the geographical range of species that rely on one another (Thomas *et al.*, 2004; Schweiger *et al.*, 2008). Additionally, distribution shifts in response to climate change, and global trade, can cause the invasion of 'alien' species (Walther *et al.*, 2009; Vilà *et al.*, 2011; Bellard *et al.*, 2013). There is compelling evidence for the disruption of plant-pollinator communities as a result of alien species which outcompete native species and spread disease (Lopezaraiza-Mikel *et al.*, 2007; Dohzono and Yokoyama, 2010; Kaiser-Bunbury *et al.*, 2011; Pyšek *et al.*, 2011).

In summary, the overall picture is of threatened pollinators, threatened plants and the potential for severe consequences. Pollinators are declining; the flowering plant species and crop species associated with them are under threat, directly and indirectly; and the ecosystems that rely on pollination are at risk. A broad spectrum of known and possible causes has been identified. Crucially, in order to protect and conserve both plants and pollinators it seems appropriate to take a system wide approach to understanding the dynamics and vulnerabilities or robustness of plant pollinator communities as a whole.

Studies that have focused on single species, specific interactions and single issues are of course valuable in teasing out the details, and there are many examples of this approach in the literature discussed so far. In this thesis we adopt a networks approach to understanding plant-pollinators communities and the complex web of interactions involved.

1.6 Network theory

Network theory enables us to visualise, quantify and analyse systems of interacting agents. A network (or graph) is any given set of pairwise interactions, between a set of agents, which make up a system. A network can be visualised as set of nodes which represent agents, connected by a set of edges which represent interactions, (See Figure 1.6). More information, specific to the system in question can be included in the network. For example, it is common to add attributes that describe something about the nodes or weight the edges according to some measure of the interaction.

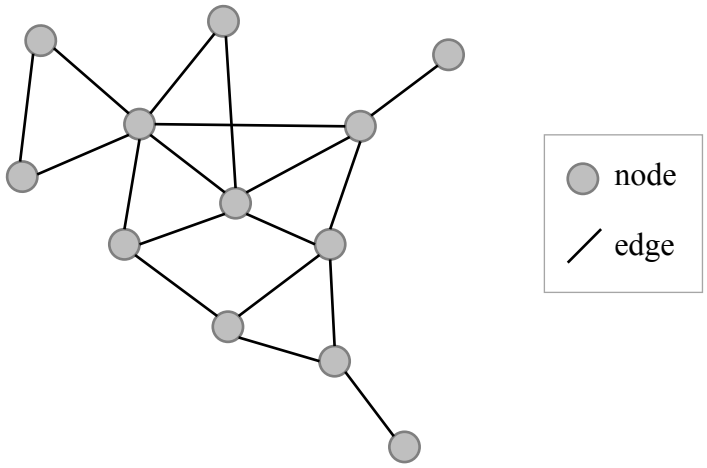

Figure 1.6 A network. The nodes (grey circles) represent agents that are connected by edges (black lines) that represent interactions.

Much of the development of network theory has been in the social sciences (see Borgatti *et al.*, 2009 for a review of network analysis in the social sciences). However, networks can be used to examine a wide variety of systems including, biochemistry and metabolic pathways (reviewed by Xia *et al.*, 2004), transport systems (reviewed by Dougherty, 1995) and infection (reviewed in Newman, 2002). The fundamental value of a

network approach to any problem, is that it allows us to examine complex relationships at a system wide level. In 1973, Robert May published a highly influential paper on "Stability and Complexity in Model Ecosystems" (May, 1973). This is regarded by some as a key moment in the development of the study of ecology (Gravel, Massol and Leibold, 2016) and network theory has increasingly been applied to ecological problems since. This is unsurprising as the very nature of ecology, the study of the interrelationships between organisms and their environment, lends itself to a network approach. Ecological networks allow us to further our understanding of organisms within a population and the environment.

Ecological networks in current research can broadly be divided into three types (Ings *et al.*, 2009). The first, and oldest are food webs. A food web is simply a network that describes the transfer of energy through an ecosystem (Hall and Raffaelli, 1993). The nodes usually represent species, and the edges represent the transfer of energy via consumption. The study of food webs arose independently from network theory, but food webs are increasingly discussed in network terms and were a key part of the early development of ecological network theory (Ings *et al.*, 2009). The second type of ecological networks are host-parasitoid networks. These describe the relationships between host species and parasites (see for example Laliberté and Tylianakis, 2010; Henri and Van Veen, 2011). The third type, and the type that we are specifically interested in are mutualistic networks. These describe mutualistic interactions between organisms. Nodes can represent individuals, species or groups and edges represent beneficial interactions between species. Mutualisms have been widely studied in behavioural ecology but only recent been considered from a network perspective (Ings *et al.*, 2009).

1.7 Plant pollinator networks

In this thesis we use a network theory approach to understanding plant-pollinator communities as mutualistic networks. Plant-pollinator networks are a well established type of mutualistic network (Ings *et al.*, 2009). Typically, the nodes in the network are of only two types; plants and pollinators (see Figure 1.7), though they can be combined with other types of mutualistic interactions as shown by Pocock, Evans and Memmott, (2012). Note that it is possible for the interaction between a plant a pollinator to not be, or only partially be mutualistic, so we use this term (as others do) as a generalisation. Plants and pollinators represent two different trophic levels; plants are primary producers and pollinators are primary consumers. Nodes can be weighted by the abundance of species. From a pollinator node to a plant node, the edge represents a feeding relationship (usually based on

observational data) where the pollinator feeds on the plant. From plant to a pollinator, the interaction represents a pollination relationship which is based on the assumed potential for pollination of the plant by the pollinator. Typically, these interactions are not separated but represented by a single edge that indicates a mutualism; pollinators benefit from the interactions in terms of the food and plants benefit in terms of potential pollination. Edges can be weighted by the abundance of interactions, or a quantitative measure of the mutualism (the latter is rare, though see Traveset, Tur and Eguíluz, 2017).

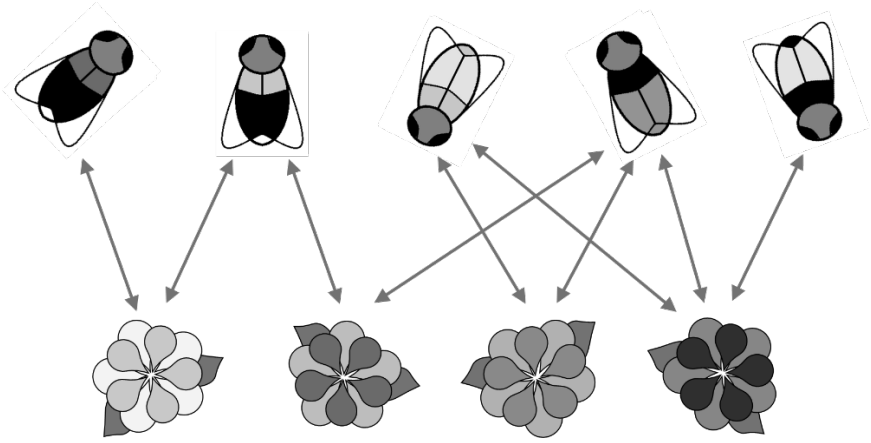

Figure 1.7 An imagined plant pollinator network showing the interactions between five pollinator species (top row) and four plant species (bottom row). In this example the edges joining plants and pollinators have an arrow at both ends to indicate that the interactions are mutalistic (beneficial to both plant and pollinator), though often in plant-pollinator network visualisations, the arrow heads are omitted.

As is often the case in ecology, observation and descriptive studies have formed the basis of our understanding of plant-pollinator communities. There is an extensive history of observational recording of the relationships between plants and pollinators which long predates the use of networks. For example, the very detailed observational records of insects visiting plants in the Rocky Mountains of Colorado published in 1923 (Clements and Long, 1923) and the more general but long term phenological records from the Marsham estate in Norfolk, UK that span 1736-1947, and can be used to infer co-existence of species (Sparks and Carey, 1995). Much of our knowledge of the basic biology of plants and pollinators is based on observations of species in their natural environment. By observing and describing, scientists have built up a foundation of knowledge about what organisms are present where, what they do and how they interact. Known relationships between plants and pollinators can be used to build a network that describes a hypothetical plant-pollinator community, as is

done by Campbell *et al.*, (2010) in their model of plant-pollinator network community assembly. More typically, plant-pollinator communities are observed in the field with the specific aim of describing the network of interactions and then exploring the network properties of these interactions. For example, Memmott, (1999) sampled a plant-pollinator community by walking fixed transects and recording observed interactions in order to determine the structural properties of the resulting plant-pollinator network (see Figure 1.8). This particular network is commonly cited and many others followed in producing plant-pollinator networks in a similar fashion (for example; Dicks, Corbet and Pywell, 2002; Olesen, Eskildsen and Venkatasamy, 2002; Bartomeus, Vilà and Santamaría, 2008; Kaiser-Bunbury *et al.*, 2017).

The ability to describe a plant-pollinator community as a network is certainly useful in its own right as it allows us to visualise the community as a whole and summarise a large amount of ecological information in a single object. Network visualisations can be used to great effect in communicating complex information to decision-makers, stakeholders and the general public (Pocock *et al.* 2016). However, there is also a great wealth of knowledge to be gained from the analysis of ecological networks. As the use of network theory to describe ecological communities has increased, various network measures and tools developed in other fields have been adopted, and new measures have been developed specifically to analyse ecological networks. The 'robust, informative and ecologically sound' of these tools and measures are expertly summarised and reviewed in a recent review by Delmas *et al.*, (2018). We will now briefly discuss some of these tools and measures, focussing on those that are used in this thesis.

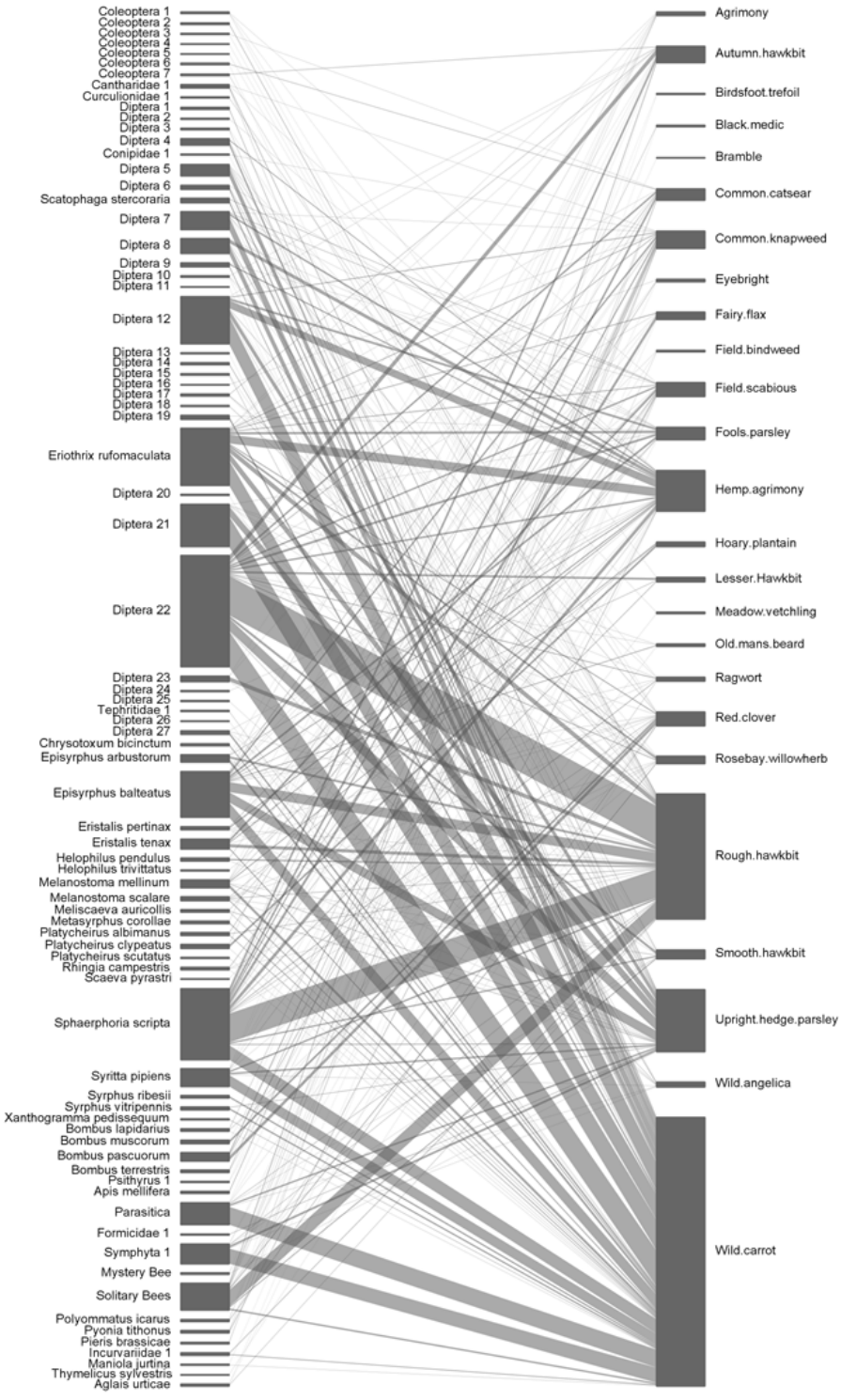

Figure 1.8 A plant pollinator network based on observations from Ashton Court in Bristol, UK (Memmott, 1999). *Pollinators are shown on the left, plants on the right. The width of the edges is proportional to the number of interactions. The height of the pollinator blocks (nodes) indicate their relative frequency as flower-visitors and the height of the plant blocks indicate the importance of the species (in terms of number of interactions) as a resource for pollinators at this site.*

Perhaps the most basic measures of a network are size and connectance. Size tells us the number of nodes; in the case of plant pollinator networks the number of plants and pollinators. Connectance is a measure of the number of interactions in the network as a proportion of the total possible interactions (Martinez, 1992). Though simple, these measures are important in defining and comparing networks. Degree distribution is a measure that describes the degree (the number of nodes a given node interacts with) of all of the nodes in a network. This has been used to identify key species in ecological networks and determine which species are generalists or specialists; species that interact with a large or small number of other species (see Sole & Montoya, 2001; Dunne *et al.,* 2002; Memmott, Waser and Price, 2004). Diameter and shortest path length can be useful measures for determining the potential impact of one species on another and have been used to look at disease spread in plant-pollinator networks (Lundgren & Olesen, 2005; Minor *et al.*, 2008). Shortest path length is the shortest path between any two nodes and diameter is the longest of all of the shortest path lengths (Albert & Barabasi, 2002). Nestedness is a measure often discussed in the analysis of ecological networks (see for example Burgos 2007; Nielsen and Bascompte, 2007; Bastolla *et al.,* 2009; Joppa, 2010). Ecological networks tend to be nested; small divisions of the network are subsets of larger divisions of the network. For plant-pollinator networks, this means that specialist pollinators tend to visit a small number of highly connected plant species, while generalist pollinators visit a large number of plant species, including those that are only visited by a small number of pollinators (Bascompte *et al.,* 2003). There are various measures of nestedness available. One of the most common, and the one we use in this thesis is that of NODF proposed by Almedia-Neto (2007). However we note that the significance of nestedness in ecological networks has been contested (James, Pitchford and Plank, 2012; Staniczenko, Kopp and Allesina, 2013). Other common network measures include; centrality (reviewed in Landherr, Friedl & Heidemann 2010) which has been used to measure the influence of species (Martin *et al.,* 2010); clustering and modularity (Watts & Strogatz, 1998), used to identify tightly connected groups of species (Marcoux & Lusseau, 2013; Gauzens *et al.,* 2015) and motifs (Milo, 2002; Simmons *et al.*, 2018), used among other things to examine species roles in networks (Baker *et al.,* 2014).

Using the various network measures described above, it is possible to compare networks and crucially move from descriptive analysis to predictive analysis. One approach is to compare network measures across multiple networks, either from different habitats, across spatial gradients or over time. For example, comparing plant-pollinator networks sampled along the chronosequence (sites of difference ages) of a glacier front to determine

how succession impacts network structure (Albrecht, Riesen and Schmid, 2010) or comparing plant-pollinator networks from different islands to determine the impact of non-native species on the network (Olesen, Eskildsen and Venkatasamy, 2002). A key issue that has been identified with this approach is that many network properties are highly correlated, and many measures are driven by connectance (as shown by Vermaat, Dunne and Gilbert, 2009; Poisot and Gravel, 2014; Chagnon, 2015) so care must be taken (discussed in Delmas *et al.*, 2018). An alternative approach is to use null hypothesis testing, where an observed network is tested against null networks. Null networks can be generated for this purpose by randomising the observed network under some pre-determined constraints (see Chapter 5 in Croft *et al.*, 2008 for a critical overiew), or by building networks according to a set of ecologically informed rules of assembly (Bascompte *et al.*, 2003; Fortuna and Bascompte, 2006). In this thesis we will we use the former approach in various different analyses.

Another approach that can be used to analyse plant-pollinator networks is by means of theoretical models. One branch of theoretical modelling popular in recent years is that of robustness. In the literature, robustness modelling falls into two main categories. In the first (see for example; Bastolla *et al.*, 2009; James, Pitchford and Plank, 2012), the network is treated as a dynamical system where mathematical equations describe parameters such as intrinsic growth, intraspecific and interspecific competition and the benefit of mutualistic interactions (based on empirical network data). The dynamics can be run to fixation or equilibrium and used to determine robustness. The second kind, which we focus on in Chapter 2, simulates extinctions by removing nodes from a plant-pollinator network and measuring robustness as the proportion of species that remain connected in the network. We will refer to these as 'knockout extinction models' to differentiate them from other types. Knock-out extinction models were first used to measure robustness in multitrophic food webs (Dunne, Williams and Martinez, 2002) and have more recently been developed further in the analysis of plant-pollinator networks. For example, compare a simple model by Memmott, Waser and Price, (2004) in which pollinator species were sequentially removed from the network (until all had been removed) and at each step the proportion of plant species that remained connected was used to determine robustness, and the more complex model by Kaiser-Bunbury *et al.*, (2010) in which plants and pollinators were removed but in each step the network was able to 're-wire' according to empirical evidence of interactions between species. In a more recent study by Traveset, Tur and Eguíluz, (2017) empirical data informs the dependence of plant species on pollinator species. In general, the trend is for more 'ecologically realistic' models of robustness. However, in Chapter 2 we will address

the largely neglected impacts of model choice and how robustness models often generate a large range of possible robustness values.

The wealth of plant-pollinator network data is increasing. Many plant-pollinator networks such as the Ashton Court network in Figure 1.8 are freely available from online databases such as the Web of Life ecological networks database (http://www.web-of-life.es). This has led to these networks being used in new studies and meta-analyses. However, there are a couple of key issues to recognise with plant-pollinator networks based on sampled data. Firstly, a network is only a sample of a real community and ecosystem. In ideal terms, it is representative but that is not always the case. Secondly, the survey methods can be very varied. Some plant-pollinator interactions are surveyed by walking a transect, others by fixed observations of individual plants. Surveys can be carried out by a lone observer or a team of people sampling at the same time. Sampling techniques influence the structure of plant-pollinator networks as shown by Gibson *et al.*, (2011) and so two networks surveyed with different methods may not be comparable. Thirdly, the time period that the network represents can vary greatly. This issue in particular is one that we will continuously revisit throughout this thesis. Some networks are a result of intensive, short-term surveying, maybe over a few days. These are often referred to as 'snapshot networks', as they are a static view of the community at a particular moment in time. Other networks are the result of aggregated, or 'lumped', data from multiple sampling periods. This might be the result of sampling several times throughout a season or sampling the same period over many years. These networks are more of a representation of the network overall through time but not at a given point. It is possible that species which were never present at the same time in the field are both present in the network giving a false impression of their co-existence. These are an example of 'forbidden links' and are recognised as a common issue with time aggregated network data (Jordano, 2016). The availability of plant-pollinator network data is a positive step in this field, but care must be taken when using this type of data (Ballantyne, Baldock and Willmer, 2015; CaraDonna *et al.*, 2017).

1.8 Motivation for this thesis

Plants and pollinators are vital to healthy ecosystems. Unfortunately, plant-pollinator communities are under pressure from a range of external threats. In order to protect and support these communities it is vital that we understand their ecology and how they respond to their environment. A network approach allows us to examine plant pollinator communities

as a whole and understand more about species within the context of their shared environment.

We have identified gaps in our current understanding of plant-pollinator communities that we seek to address in this thesis. Firstly, we are interested in exploring plant-pollinator networks over a range of timescales. As noted by Alarcón, Waser and Ollerton (2008), Blüthgen (2010) and CaraDonna *et al.*, (2017) few studies have addressed the impacts of temporal variation in plant-pollinator networks and Delmas *et al.*, (2018) suggest the comparison of networks over time as a key area for development. We are particularly interested in temporal variation within the context of sampling networks. This is motivated by the use of plant-pollinator networks without consideration of temporal sampling effects. Snapshot networks only represent the period of time over which they were sampled and so may over-emphasise the importance of species that were abundant for only a short period while aggregated networks may contain forbidden links and miss important details of temporal variation. The second gap we are interested in addressing is the lack of experimental studies on plant-pollinator networks. So far, studies of ecological networks have largely involved comparative or theoretical analysis of observational data. This is not surprising considering the complexity of manipulating an ecological network in its natural environment and measuring outputs. These studies can be time consuming and labour intensive.

The overarching aim of this thesis is to further our understanding of plant pollinator communities with regards to temporal changes using theoretical, observational and experimental techniques. We first examine theoretical modelling techniques that use observed plant-pollinator network data to predict the effect of the loss of species over time (Chapter 2). Considering the timescales of flowering plants, and pollinator lifecycles it is clear that the floral environment changes over time and across years and that pollinators must be able to adapt to these changes in order to survive (as we show in Chapter 3). We then consider how pollinators adapt to changing resource availability and hence impact plant-pollinator interactions. Specifically, we consider the impact of manipulating floral abundance with a pulse perturbation (removal and regrowth) experiment (Chapter 4). Finally, we explore the foraging patterns of bumblebees via focal follows of individuals (Chapter 5).

1.9 Overview of key objectives and thesis layout

Chapter 2

Key Question: How is robustness of plant-pollinator networks (calculated using knock-out extinction models) impacted by model mechanism and the structural properties of the network?

Records of declines and extinctions across a range of species suggest that plant-pollinator communities are vulnerable as a whole. Because the relationships in a plant-pollinator community are mutualistic, the decline or loss of one species is likely to have a knock-on effect on other species that it interacts with. Models of community robustness, specifically knockout extinction models, are one way of predicting changes to the community over non-specific but long ecological timescales (long enough for extinctions to occur). In recent years, various studies have proposed improved knockout extinction models with an emphasis on more biological realism. However, less attention has been given to understanding how the mechanism of these different models, and the structure of the plant-pollinator community analysed, impacts the reported robustness. In Chapter 2, we present a suite of knockout extinction models, the outputs of which can all be directly compared. Direct comparison is crucial, as it allows us to determine the effect of model mechanism on robustness. We also show how the structure of the plant-pollinator network being analysed affects robustness, particularly with respect to highly abundant and well-connected plant species. Overall, Chapter 2 is about quantifying the robustness of a plant-pollinator community to extinctions and understanding the output of robustness models.

Chapter 3

Key Question: How does the floral resource in a plant-pollinator community change within a pollination season, and how different are two consecutive seasons in this regard?

The floral resources available to pollinators change over a time as different plant species flower at different times and for varying periods. This concept is well understood, but there is a lack of detailed data that covers the whole flowering period of different species; many phenological records focus on 'first flowering date' only. In Chapter 3 we present phenological data from 3 adjacent habitats, that describe the flowering phenology of 60 plant species from March to September in 2016 and 2017. Crucially, this data shows what floral resources were available to pollinators throughout the season. We quantify plant species turnover, compare the phenology of plant species within season, across sites and across the two years and demonstrate the succession of highly abundant plant species. We

also present records of bumblebee species interacting with plant species in the three habitats during the survey periods. This gives us a coarse-grained account of when bumblebee species are foraging and how their main floral resources change over time. The main purpose of the work in Chapter 3 was for us to understand the variation in flowering plant species over a season and across two consecutive years at the site and how this impacts the community. Additionally, this study enabled us to plan and set the scene for an experimental manipulation of the floral resources at the site, which we present in Chapter 4.

Chapter 4

Key Question: What is the impact of the experimental removal of a plant species from a plant-pollinator network in terms of network structure, the foraging choices of bumblebee species, and knock on effects to other pollinators?

Floral resources change, as a result of normal temporal dynamics, and unexpectedly as a result of external threats. Plant-pollinator communities must adapt to these temporal changes in order for species to survive and continue functioning as a community. Theoretical techniques, such as the robustness models in Chapter 2, are one way of predicting the effect of species loss on a plant-pollinator community. However, they are based on relatively simple assumptions about the dynamics and flexibility of the community and simulate changes over a non-specific, long timescale. There is a commonly reported scarcity of empirical, experimental data to inform and back up theoretical techniques in this area of research. In Chapter 4 we present a 'pulse-perturbation' experiment in which we removed the flowers of an abundant plant species from one of the habitats discussed in Chapter 3, and then allowed the plant to re-flower. Our aim to was to determine the impact of experimental species removal on a plant-pollinator community in terms of the network and the behaviour of abundant pollinator species (we focus on 4 abundant bumblebees), as well as the ability of the community to 'heal' after the re-flowering of the plant species. Overall, Chapter 4 presents evidence for the network response and bumblebee species response to the manipulation of a plant species.

Chapter 5

Key question: How constant or prone to switching are foraging bumblebees over consecutive flower visits in a resource rich environment and are there differences in foraging between species?

To complete this thesis, we focus on the foraging behaviour of individual bumblebees and how foraging patterns could contribute to a bumblebee colony's ability to adapt to a changing floral environment over longer timescales. Studies of optimal foraging behaviour suggest that a bumblebee individual should preferentially forage on one species of

plant that is abundant and high reward in terms of pollen and nectar. However, bumblebees must switch between plant species in order to adapt to changes in floral resources overtime, as shown in Chapters 3 and 4. In Chapter 5 we present focal follow observations in which we track individual bumblebees, of 4 abundant species, for 5 consecutive flower visits in a flower-rich meadow. We compare observations from June and July at the same site. We describe bumblebees that feed on only one plant species as being 'flower constant'. We quantify the level of constancy for each bumblebee species using a model that takes into account our knowledge of the flower resources available to each bumblebee individual. We also examine floral cues; characteristics of flowers that bumblebees may use to decide what to forage on (*e.g.* colour and symmetry). We determine whether bumblebees switch between flower species based on floral cues. Overall, in Chapter 5 we aim to understand the foraging patterns of individual bumblebees in order to inform our overall understanding of how plant-pollinator communities change over different timescales to adapt to their changing environment.

Chapter 6

Conclusions

In Chapter 6 we synthesise the key results from this thesis. We tie together the threads of ideas from the separate chapters to give an overview of the thesis as a whole and present the final conclusions.

A note on thesis format

This thesis is presented in 'Alternative Thesis' format. Chapter 2 is the contents of a paper published in Ecology and Evolution in 2018 with Miranda Bane as the lead author (Bane, Pocock and James, 2018). The text and figures in the chapter are identical to the paper, though the formatting is changed to fit with the rest of the contents of this thesis. Chapters 3, 4 and 5 are unpublished work.

Chapter 2

Effects of model choice, network structure, and interaction strengths on knockout extinction models of ecological robustness

This chapter was published as a paper in journal Ecology and Evolution in October 2018. For this thesis, we have updated the section and figure heading numbers to match the rest of the thesis, but the main text and the figures are as they appear in the published paper. Therefore, there is some repeat of information covered in Chapter 1, particularly in establishing the main context and background. Other than this, the text fits within the thesis and can be read as a chapter. We have included an extra section (2.5) at the end of the discussion to tie together the main conclusions of the paper with the overarching aims of the thesis. The supplementary information from the paper is included as an Appendix at the end of the chapter.

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This declaration concerns the article entitled:									
Effects of model choice, network structure, and interaction strengths on knockout extinction models of ecological robustness									
Publication status (tick one)									
Draft manuscript		**Submitted**		**In review**		**Accepted**		**Published**	✓
Publication details (reference)	Bane, M.S., Pocock, M.J. and James, R., 2018. Effects of model choice, network structure, and interaction strengths on knockout extinction models of ecological robustness. *Ecology and Evolution*. 8(22). doi: 10.1002/ece3.4529								
Candidate's contribution to the paper (detailed, and also given as a percentage).	The candidate (M. Bane), R. James and M. Pocock contributed equally to the formulation of ideas. M. Bane contributed to the design of the methodology (30%) with R. James (40%) and M. Pocock (30%). M. Bane coded the models and carried out the analysis of the data (80%) with support and technical advice from R. James and M. Pocock, and input from W. Bonnell in developing the Random Walk model (recognised in acknowledgements). M. Bane contributed considerably to the interpretation and presentation of results (70%) with support from R. James and M. Pocock. M. Bane drafted the manuscript to be sent for publication (60%), with critical feedback and guidance from R. James and M. Pocock. M. Bane responded to feedback from reviewers in discussion with R. James and M. Pocock and all three authors gave their final approval for submission.								
Statement from Candidate	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.								
Signed						**Date**			

Abstract

Analysis of ecological networks is a valuable approach to understanding the vulnerability of systems to disturbance. The tolerance of ecological networks to coextinctions, resulting from sequences of primary extinctions (here termed "knockout extinction models", in contrast with other dynamic approaches), is a widely used tool for modelling network "robustness". Currently, there is an emphasis to increase biological realism in these models, but less attention has been given to the effect of model choices and network structure on robustness measures. Here, we present a suite of knockout extinction models for bipartite ecological networks (specifically plant–pollinator networks) that can all be analysed on the same terms, enabling us to test the effects of extinction rules, interaction weights, and network structure on robustness. We include two simple ecologically plausible models of propagating extinctions, one new and one adapted from existing models. All models can be used with weighted or binary interaction data. We found that the choice of extinction rules impacts robustness; our two propagating models produce opposing effects in all tests on observed plant–pollinator networks. Adding weights to the interactions tends to amplify the opposing effects and increase the variation in robustness. Variation in robustness is a key feature of these extinction models and is driven by the structural heterogeneity of nodes (specifically, the skewness of the plant degree distribution) in the network. Our analysis therefore reveals the mechanisms and fundamental network properties that drive observed trends in robustness.

2.1 Introduction

Network analysis has become an important tool for ecologists seeking to understand the vulnerability of ecosystems to natural and anthropogenic disturbance. Recent research has centred on network approaches for improving our understanding of plant–pollinator communities and extinctions, especially in the light of the widely documented declines in key insect pollinators such as honeybees, bumblebees, and butterflies (Benton, 2006; Biesmeijer, 2006; Goulson, Lye and Darvill, 2008; Senapathi *et al.*, 2015). These trends are concerning for biodiversity, ecosystem function, and food security (Potts *et al.*, 2016) as insect pollinators play a vital role in providing ecosystem services (Bailes *et al.*, 2015). They feed on nectar and pollen provided by plant species, and whilst doing this, facilitate the fertilization of plants via cross-pollination (Lubbock, 1897; John Brand Free, 1970). In plant–pollinator systems, the community can be regarded as a bipartite network comprising two distinct guilds of organisms in which each node represents a species, and species are connected by edges indicating interactions, which may be directly observed, indirectly observed (e.g., pollen analysis), or inferred (Morales-Castilla *et al.*, 2015).

Models of community robustness based on observed plant–pollinator networks (available, e.g., from: http://www.web-of-life.es and https://www.nceas.ucsb.edu/interactionweb/resources.html) usually fall into one of two types. In the first (see for example Bastolla *et al.*, 2009; James, Pitchford and Plank, 2012), the community is modelled as a dynamical system, in which the population of each species is affected by the interactions that each species has with others. The dynamics are typically run to fixation, and the populations at fixation used to determine community robustness.

The second approach, adopted here, is to model the tolerance of the network to simulated extinctions (henceforth "knockout extinction models"). In ecology, this approach was applied first to multitrophic food webs (Dunne, Williams and Martinez, 2002) and then mutualistic bipartite networks, especially plant–pollinator networks (Memmott, Waser and Price, 2004; Kaiser-Bunbury *et al.*, 2010). Campbell, Yang, Shea, and Albert (2012) use a very similar approach to study the effects of forced species extinctions. The networks they analyse differ from those considered here, in that they are all generated by a (dynamic Boolean) model of plant–pollinator community formation (Campbell *et al.*, 2010).

Knockout extinction models estimate the robustness of a plant– pollinator network by sequentially removing species of the primary type (e.g., plants) and recording the number of surviving species of the secondary type (e.g., pollinators), by applying some

predetermined rule for species survival. Network robustness can then be determined from the area under the curve of the proportion of the secondary type that survive against the proportion of the primary type removed (Burgos *et al.*, 2007; see Figure 2.1).

In the simplest, "Secondary Only" (SO) knockout models, primary extinctions from one guild lead only to secondary extinction of species in the other guild. Primary extinctions are chosen in a specific order—determined by the number of interactions a species has, for example—or in a random order (Dunne, Williams and Martinez, 2002; Memmott, Waser and Price, 2004; Pocock, Evans and Memmott, 2012). A key development by Vieira and Almeida-Neto (2015) was to allow coextinction due to feedback between guilds, so permitting cascades of extinctions. The propagating extinction model of Traveset, Tur, and Eguíluz (2017) incorporates empirically estimated dependencies of plants on pollinators. In a different development, Kaiser-Bunbury et al. (2010) allowed edge rewiring (pollinators switching from one plant to another) based on empirical evidence; others have explored robustness to edge, not node, knockouts (Valiente-Banuet *et al.*, 2015; Santamaría *et al.*, 2016).

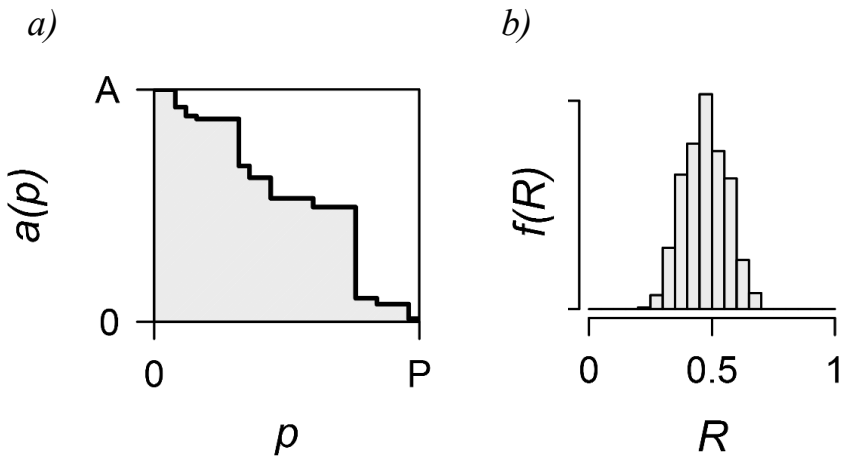


Figure 2.1 The output of a knockout extinction model. ***a)*** *For a single extinction sequence, the number of surviving pollinator nodes a reduces as the number of plant nodes made extinct, p, increases until a = 0. Robustness (R) = 0.550 is the area under a(p), divided by the area of the rectangle, AP.* ***b)*** *In all our extinction models, the value of R depends on the order in which plants are made extinct, so many simulations of random sequences of primary extinctions are used to produce a distribution of robustness values f(R)*

SO models were used to show that the robustness of communities to random primary extinctions increases with network connectance, that is the fraction of the possible interactions that were actually observed (Dunne, Williams and Martinez, 2002) and the

resulting robustness was often interpreted in terms of network nestedness (Memmott, Waser and Price, 2004). Vieira and Almeida-Neto (2015) found that cascades were more likely in highly connected networks. However, more detailed investigation of the impact of network structure on robustness has been lacking.

Most early empirical plant–pollinator networks were binary; interactions between pairs of species were either observed or not. However, researchers are increasingly measuring the frequency or importance of interactions to create weighted networks, yielding a better description of the interactions observed (Memmott, 1999; Ings *et al.*, 2009), and accounting better for under-sampling biases (Banašek-Richter, Cattin and Bersier, 2004). More recent models have used weighted data in different ways: using node abundance to weight the binary outcomes (Kaiser-Bunbury *et al.*, 2010) or using empirically determined, weighted dependences of plant species on pollinators (Traveset, Tur and Eguíluz, 2017).

One of the features of knockout extinction models is that, when using random sequences of primary extinctions on a single empirical network, there is a broad distribution in the resulting robustness values (Figure 2.1). Robustness must therefore be a product both of structural heterogeneity of the network (e.g. Pastor *et al.*, 2012) and of the method of producing extinction sequences.

The aim of this paper was to understand in detail which features of knockout models, and which properties of empirical ecological networks, are responsible for the central value and range of computed robustness distributions. To this end, we bring together a suite of models—a simple SO model and two simple propagating extinction models—and use them to compute the robustness of a number of empirical plant–pollinator networks in both binary and weighted form. The models were chosen for their simplicity and direct comparability, not to achieve ecological realism.

2.2 Materials and methods

In this study, we examine the robustness of observed plant–pollinator networks that describe observed interactions between species in a community. A network has P plant nodes and A animal nodes, and contains E interactions between species, encoded in the $A \times P$ matrix $\underline{\underline{M}}$. Interactions may be binary (b) or weighted (w). We illustrate our models and findings using a plant–pollinator network, collected by Memmott (1999), from Ashton Court, a site in Bristol, UK. We will refer to this as the Ashton Court (AC) network. This is a well-sampled network (Blüthgen, Menzel and Blüthgen, 2006) with interactions recorded

over a short period of time (1 month). The AC network is highly resolved: all plants were identified to species (P = 25) and pollinators (A = 79) mostly identified to species level (morphotyped otherwise). $\underline{\underline{M}}_{AC}$ contains 104 species, E = 299, with connectance (proportion of realized interactions) c = 0.151 and nestedness (Almeida-Neto *et al.*, 2008) NODF = 42.5%. Interactions in the AC network are weighted by the number of observed visits of each pollinator species to each plant species. The plant degree distribution is highly skewed, with a high proportion of pollinators visiting a single plant species, as is often the case in plant–pollinator networks.

For comparison, we also present results for five other networks. We selected networks (summarized in Table 1) that had weighted edges (by visits), were well resolved, had $P > 12$, had a range of c and NODF, and for which, we had access to collection methods.

Table 2.1 Summary of the networks used in this paper. 1: (Memmott, 1999), 2: (Small, 1976), 3: (Kaiser-Bunbury et al., 2010), 4: (Dicks, Corbet and Pywell, 2002), 5: (Dicks, Corbet and Pywell, 2002), 6: (Bartomeus, Vilà and Santamaría, 2008)

Network	**Plants** (P)	**Pollinators** (A)	**Interactions** (E)	**Connectance** (c)	**Nestedness** (NODF)	**Largest P degree**
Ashton Court[1]	25	79	299	0.151	42.54	49
Ottawa[2]	13	34	141	0.319	40.96	18
Mauritius[3]	14	24	46	0.137	18.30	7
Shelfanger[4]	16	36	85	0.148	35.66	21
Hickling[5]	17	61	146	0.141	52.27	49
Creus[6]	32	81	319	0.123	28.01	28

2.2.a Model development

We took as our starting point the extinction model of Memmott et al. (2004), who analysed the robustness of binary networks by making species of one type (in their case, pollinators) extinct in a random order, that is they used a random primary extinction sequence. From this, we developed two new extinction models, with differing ecological

bases, that each includes sub-sequences of plant extinctions determined by network structure. All three models (summarized in Figure 2.2) use either edge weight or (binary) edge existence to decide secondary (and further) extinctions. In this section, we first describe the features that are common to all our extinction models and then outline the distinctive features of each, providing the ecological context and highlighting the relationships between ours and previous knockout extinction models.

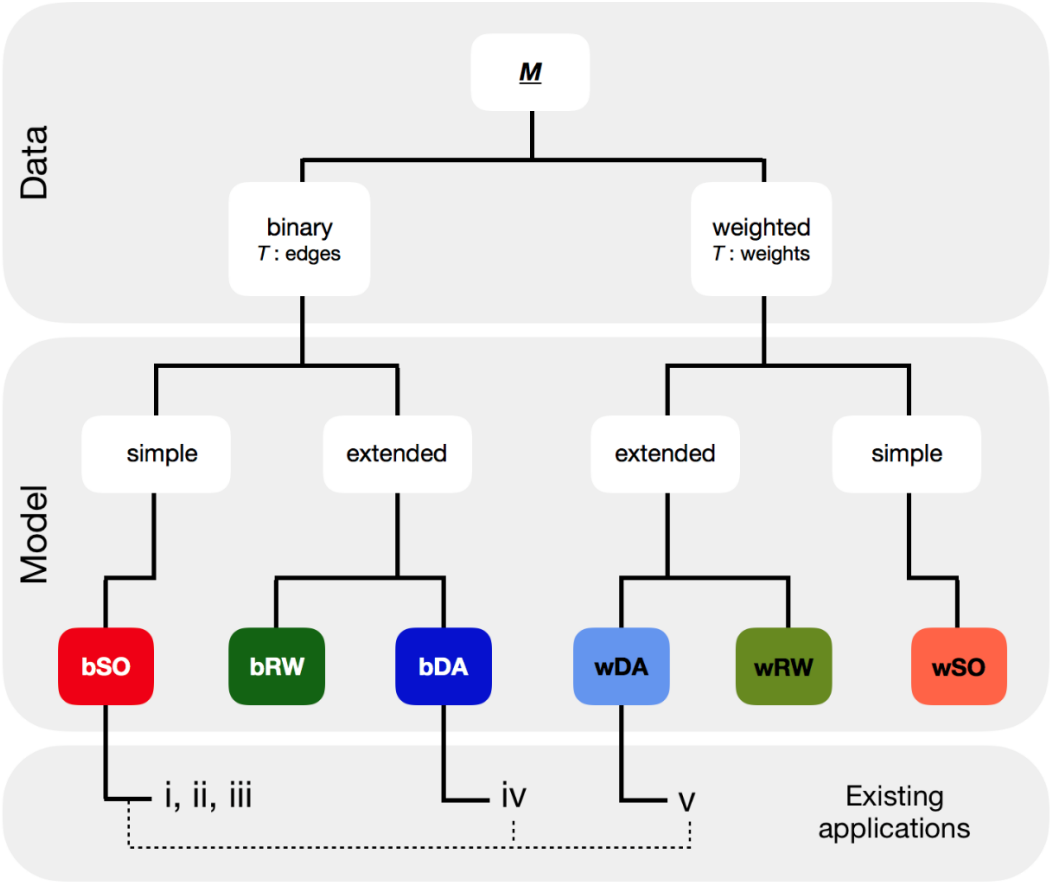


Figure 2.2 Framework of extinction models, with those used in this study highlighted in colour. All models start from an observed bipartite network M that can be binary (prefix b) or weighted (w). For binary data the threshold T is applied to the number of edges; for weighted data it is applied to the weights. Models are split into those that produce entirely random primary extinction sequences: Secondary Only (SO), and those that introduce other methods for determining extinction sequence: Deterministic Avalanche (DA) and Random Walk (RW). (i-v) indicate previous studies that represent special cases of the models in the framework where (i-iii) T = 1: i) Dunne, William and Martinez, 2002; ii) Memmott, Waser and Price, 2004; iii) Kaiser-Bunbury et al. 2010, iv) where T is applied stochastically and extinctions can 'cascade' (Vieira and Almeida-Neto, 2015) and. v) a hybrid of iv) and bSO with empirical plant dependencies (Traveset, Tur and Eguíluz, 2017).

2.2.b Universal model features

Starting from the observed matrix $\underline{\underline{M}}$, a node of one guild (plants) is removed as a primary extinction. Extinctions result in the loss of interactions from $\underline{\underline{M}}$, monitored in the "reduced" matrix $\underline{C}$. The loss of interactions may, according to the rules of the particular model, result in the secondary loss of nodes of the other guild (pollinators). In our new models (see below), the rules admit the possibility of each secondary pollinator extinction giving rise to further knock-on plant extinctions, further pollinator extinctions, and so on. Any such plant extinctions cannot be considered "primary", but will take their place in what we shall continue to refer to as a "primary extinction sequence" of the P plant species. All models proceed until all plant nodes are removed and all species - plants and pollinators - are extinct, as in previous studies. The robustness (R) of the network is calculated as

$$R = \frac{1}{AP}\sum_{p=0}^{P} a(p),$$

where p is the number of plant species that have gone extinct (from 0 to P) and $a(p)$ is the number of pollinator species remaining in the network (from A to 0). R is the normalized ($0 < R < 1$) area under the curve of a graph of the proportion of plant nodes that have gone extinct against the proportion of surviving pollinator nodes (Figure 2.1). Values of R closer to 1 indicate higher "robustness" of the network to primary extinctions (e.g. Burgos et al., 2007). We use $a(p)$ as our response variable for all models in order to facilitate comparisons, although we note other options are possible: Kaiser-Bunbury et al. (2010) used the sum of interaction weights $w(p)$. The value of R is dependent on the specific sequence of primary extinctions, so running many random extinction sequences will, for all our models, produce a frequency distribution of values of R (Figure 2.1) which we denote $f(R)$. A key model feature we adopted, one that is necessary to put all models on an equal footing, and thereby to enable fair comparison between them, is a threshold rule for secondary extinctions: A node becomes extinct once it has lost a fraction T or more of its observed interactions (binary $\underline{\underline{M}}$), or of its observed total interaction weight (weighted $\underline{\underline{M}}$). Clearly, the value of T that we choose is arbitrary. It must lie in the range $0 < T \leq 1$. [$T = 0$ is an uninformative case; all pollinator species become extinct after the first primary plant extinction; $T = 1$ generates the extinction rule for most previous models (Dunne et al., 2002; Kaiser-Bunbury et al., 2010; Memmott et al., 2004), although Vieira and Almeida-Neto (2015) introduced a node specific threshold ≤1.] We generated distributions of robustness $f(R)$ for a range of threshold values (0.1 to 1 at 0.1 intervals) for six observed plant–pollinator networks (summarized in

Table 1) to determine the effect of T on R. We then chose a threshold of $T = 0.5$ for all nodes for the remainder of this chapter: that is, a secondary extinction occurs when a node has lost at least half its interactions (binary $\underline{\underline{M}}$) or weights (weighted $\underline{\underline{M}}$). It should be noted that the "effective threshold" (T_{eff}) could be greater than T; for example, with a binary network and $T = 0.5$, a node linked to five others would go extinct after losing three edges, giving an effective T of 3/5 = 0.6. Since most pollinators are observed visiting a relatively small number of plants, the difference between the specified and the "effective threshold" can be noticeable; we report the node-averaged T_{eff} in all cases.

2.2.c New extinction model features

We present three distinct models, which we denote: 1. Secondary Only (SO), 2. Deterministic Avalanche (DA), and 3. Random Walk (RW). Each model can be used with binary or weighted interaction data and is prefixed with "b" or "w" to indicate which. In ecological terms, SO is the most simple; the next (plant) extinction is always chosen randomly from those remaining, and all choices are independent of each other. The SO model is essentially that used by Dunne et al. (2002), Memmott et al. (2004) and others, and serves as our baseline. Its ecological premise is that a plant extinction will only affect the pollinator species that visit that plant; that is, there is a uni-directional dependence in the interactions. The DA and RW models each introduce mutualistic dependencies between the guilds, in ways that remove independence from some subsequences of plant extinctions; that is, each allows the spread of extinctions through the community network. In DA, extinctions "ripple" out from an initial extinction causing a wave of collapse, as resources (interactions) diminish for both guilds. In RW, the contagion of extinction jumps from plant to plant according to their number of shared visitors, as might occur when a plant disease is spread through the community by visiting pollinators, or a pollinator disease is spread through shared floral resources (as reported by McMahon *et al.* (2015).

Model 1. Secondary only model (bSO and wSO)

In the Secondary Only model, the order of primary plant extinctions is random. All pollinator extinctions are secondary and determined by the threshold rule. The method is as follows:

1. Select a random plant species (e) for primary extinction from those left (from matrix $\underline{\underline{M}}$ the first time, then subsequently matrix $\underline{C}$)
2. Make pollinator species connected to e extinct if they have lost a proportion $\geq T$ of their original edges (bSO) or edge weights (wSO)

3. Count the number of pollinator species remaining, $a(p)$, in the updated network (matrix $\underline{\underline{C}}$)

Repeat steps 1 to 3 until there are no species remaining. Then calculate R according to Equation 1. In the special case $T = 1$, the bSO and wSO models are identical to each other, and to the model described by Memmott et al. (2004). Kaiser-Bunbury et al. (2010) employed an adaptation to the special case $T = 1$ and used the weight of remaining edges $w(p)$ as their response variable.

Model 2. Deterministic avalanche model (bDA and wDA)

In the DA model, a randomly chosen primary (plant) extinction a 'trigger' may produce secondary extinctions (of pollinators) that themselves leave plant species with less than a fraction T of their observed interactions. If this happens, there is an "avalanche" of plant extinctions. During the avalanche, the sequence of plant extinctions is not random, but is determined by network structure. At the end of an avalanche, a new, random, trigger is chosen. The method is as follows:

1. Select a random plant species (e) for primary extinction from those left (from $\underline{\underline{M}}$ the first time, subsequently $\underline{\underline{C}}$)—this is a trigger
2. a. Make pollinator species connected to e extinct if they have lost a proportion $\geq T$ of their original edges (bDA) or edge weights (wDA)
    b. Count the number of pollinator species remaining, $a(p)$, in $\underline{\underline{C}}$
    c. Make plant species (there may be more than 1) extinct according to the threshold rule as above
3. Repeat steps 2a to 2c until there is no further spread of extinctions, then repeat from step 1 with a new trigger.

Repeat steps 1 and 2 until there are no species remaining in the network. Then calculate R according to Equation 1. Were $T = 1$ used here, step 2c would never result in tertiary plant extinctions and no avalanches would occur, so the DA and SO models would be identical. The "stochastic co-extinction model" (SCM) developed by Vieira and Almeida-Neto (2015) is a special case of the bDA model where the threshold is applied stochastically and is node specific; specifically, extinctions of nodes at our step 2c occur with probability = 1-(remaining interactions)/(interactions at start). We adopt the term "avalanche" for our spreading deterministic extinctions to differentiate them from the stochastic "cascades" of

Vieira and Almeida-Neto (2015), which occur once only, triggered by the first primary extinction. Traveset et al. (2017) employed what is essentially a hybrid SCM-bSO model, with empirical dependencies for plants and allowing only two-step cascades.

Model 3. Random walk model (bRW and wRW)

The RW model is similar to DA, in that a trigger can cause an avalanche of non-random plant extinctions. In this model, the order of plant extinctions within an avalanche is determined by the (updating) structure of the $P \times P$ matrix $\underline{\underline{F}}$ whose entry F_{eg} is the number of remaining pollinator species shared by plant species e and g. The full method is as follows:

1. Select a random plant species (e) for primary extinction from those left (from $\underline{\underline{M}}$ the first time, subsequently $\underline{\underline{C}}$)
2. Construct matrix $\underline{\underline{F}}$
3. Select the next plant extinction (f) from $\underline{\underline{F}}$. Each potential choice of plant (g) is chosen with a probability proportional to F_{eg}.
4. Make pollinator species connected to e extinct if they have lost a proportion $\geq T$ of their original edges (bRW) or edge weights (wRW)
5. Count the number of pollinator species remaining, $a(p)$, in the updated matrix $\underline{\underline{C}}$
6. Identify plant f as the new e and make it extinct
7. Loop through steps 2–6. If no neighbours exist in step 3, revert to step 1.

Repeat steps 1–7 until there are no species remaining in the network. Then calculate R according to Equation 1.

2.2.d Natural extensions of our models

We have coded these three models for application with a random order of primary plant extinctions (i.e., the selection of the next extinction in step 1 of Models 1,2 and 3 is random). The models can all easily be modified to use ordered primary extinctions, where the choice of plant in step 1 is according to a predetermined rule (based on node degree, biological plant trait etc.). The models can also be applied to bipartite networks with uni-directional dependencies (no feedback between the trophic levels, e.g., trophic or host–parasitoid interactions), though in that case avalanches cannot occur.

2.2.e Comparison of robustness distributions from the three extinction models

The distribution $f(R)$ generated from a single network $\underline{\underline{M}}$ will depend on the model used and whether the edges are weighted or binary. If there are P plant species in the network, there are $P!$ distinct plant sequences. The SO models sample uniformly from these possibilities (i.e., all sequences are equally likely). The DA and RW models do not sample uniformly, because avalanches produce non-random sub-sequences determined by the structure of the network. Using binary and weighted versions of the Ashton Court (AC) network, we generated 25,000 extinction sequences using each of the three models, in order to assess the effect of model choice on R. To create values of R that lie close to the theoretical maximum and minimum bounds, we ran bSO with plant extinctions in order of increasing and decreasing degree.

2.2.f Testing on other networks

We tested our models on five other networks (Table 1). For each network, we generated 25,000 extinction sequences, using each of the three models, in binary and weighted form. We used a fixed threshold of $T = 0.5$ for all cases because we are not directly comparing the networks, only seeking to confirm the generalities of the resulting $f(R)$ distributions.

2.2.g Assessing how node and network-level properties effect variation in robustness

The breadth of the distribution $f(R)$ appears to be large in networks, such as AC, with a large range in plant degree (see Section 3). Previous studies have hinted at the probable role of degree (k) in determining robustness (James et al., 2012; Joppa, Montoya, Vicente, Sanderson, & Pimm, 2010; but see also Blüthgen et al., 2006). We therefore constructed two tests of the effect of degree on robustness, using the AC network as a test case, under each of our three knockout extinction models.

2.2.h Robustness distributions of networks with manipulated degree distributions

Our aim here was to look at the effect on $f(R)$ of replacing one or both of the observed degree distributions ($g_A(k)$ for pollinators, $g_P(k)$ for plants) with something closer to what we would expect from random rewiring of the observed interactions; a Poisson-like distribution with a well-defined single peak, relatively small variance and few outliers.

Firstly, we constructed an ensemble of 10,000 networks in which all 299 interactions in the binary $\underline{\underline{M}}_{AC}$ were placed between a random plant and pollinator, enabling us to compute the randomized degree distribution ($\tilde{g}_A(k)$, $\tilde{g}_P(k)$) for each random network and the average (or expected) degree distributions ($G_A(k)$, $G_P(k)$). We then chose, from the ensemble, the single exemplar network whose ($\tilde{g}_A(k)$, $\tilde{g}_P(k)$) was closest to the average ($G_A(k)$, $G_P(k)$), [we minimised $\sum_k[|\tilde{g}_A(k) - G_A(k)| + |\tilde{g}_P(k) - G_P(k)|]$] and used that single network to represent a manipulated version of AC in which the 299 interactions are between random pairs of species. The key feature here is that the single chosen network has degree distributions that are unremarkable, but different from the observed. For our other two degree manipulations, we conserved the observed degree distribution of one guild, but randomized the other (by redistributing the elements in rows, or in columns of the binary $\underline{\underline{M}}_{AC}$). We again chose a single exemplar network whose degree distribution, for the randomized guild, was closest to the expected distribution for the ensemble of 10,000 random networks. For each of our three exemplar networks, we ran 25,000 simulations using bSO, bDA, and bRW. We used only one randomized network from each ensemble deliberately, the better to focus on the effect of manipulating $g_A(k)$ or $g_P(k)$ or both on the robustness distribution $f(R)$.

2.2.i Plant extinction rank and degree

To explore whether (for example) high-degree plants tend to go extinct toward the beginning of a primary extinction sequence, we recorded the position in a sequence when each plant became extinct as its extinction rank (r), $1 \leq r \leq P$. We ran each extinction model 25,000 times, using binary and weighted versions of AC, and computed $h(r)$, the distribution of extinction rank for each species generated by the simulations. We tested for correlation, using the Spearman coefficient, between a plant's median extinction rank (r_m) and degree (k). By construction, r_m should be the same for all plant species under the SO model, but not necessarily under the DA or RW models, since avalanches and random walks may tend to select (or avoid) high-degree nodes preferentially.

2.3 Results

2.3.a Varying the value of the threshold for secondary extinctions

Median robustness R_m increases monotonically but nonlinearly with T. Figure 2.3 shows (for three illustrative networks) that there is a crossover; the least robust network at

low T becomes the most robust at high T. This is an artefact of the variation of effective threshold with node degree; R_m increases linearly with T_{eff} and the three networks are increasingly robust in order of increased connectance, as found by Dunne et al. (2002), at all values of T_{eff} (Figure 2.3 for three illustrative networks; all six in Appendix: Figure 2.7). The remainder of our results are presented for the AC network where $T_{\text{eff}} = 0.694$ for our chosen $T = 0.5$.

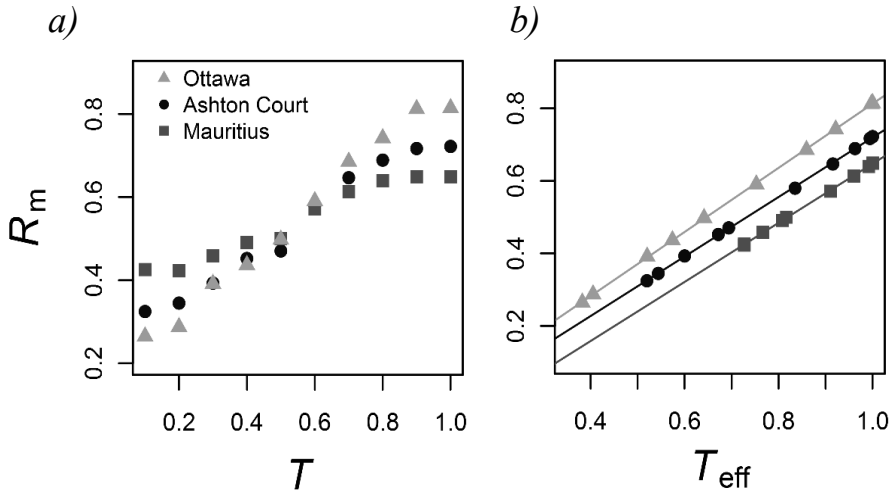


Figure 2.3 The relationship between extinction threshold (T), median robustness (R_m), and effective threshold (T_{eff}) for plant–pollinator networks: Ashton Court (triangles), Mauritius (squares), and Ottawa (circles) using the bSO model. Variation of ***a)*** *R_m with T, and* ***b)*** *R_m with T_{eff}*

2.3.b Robustness distributions for the Ashton Court network

The distributions $f(R)$ produced by each of the three models for binary and weighted data using the Ashton Court network (Figure 2.4) are all rather broad, suggesting a strong dependence of R on the order in which plants are made extinct; the computed values span the range generated by primary extinction sequences in bSO with plants removed in increasing and decreasing order of degree (R = 0.178 and R = 0.812 respectively). The bSO model produces a relatively symmetrical $f(R)$ with a median $R_{\text{m}} = 0.470$. Using the bSO model as a baseline, the bDA model shifts $f(R)$ to the right ($R_{\text{m}} = 0.512$), inferring greater robustness, and bRW strongly shifts $f(R)$ to the left ($R_{\text{m}} = 0.337$) inferring lower robustness. The same trends are shown for weighted data: $R_{\text{m}} = 0.500$ (wSO), 0.564 (wDA), and 0.321 (wRW).

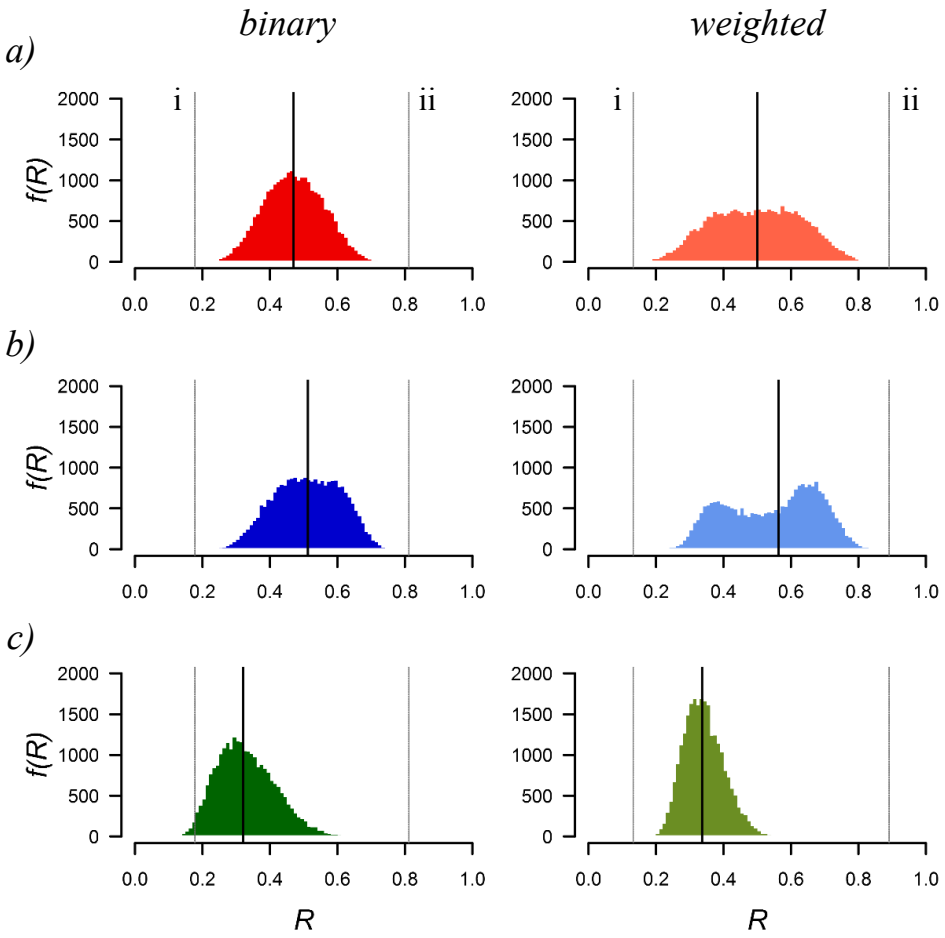


Figure 2.4 The distribution of robustness f(R) for the Ashton Court network, in binary (left column) and weighted (right column) form, generated by the three extinction models: ***a)*** *Secondary Only (SO),* ***b)*** *Deterministic Avalanche (DA), and* ***c)*** *Random Walk (RW). Median robustness R_m for each distribution indicated by the solid vertical line. (i) and (ii) indicate R values for the bSO model generated by removing plant species in increasing and decreasing degree order: b: 0.178 and 0.812, w: 0.133 and 0.891*

2.3.c Robustness distributions for other networks

The distributions $f(R)$ for the other five networks tested (see Appendix: Figure 2.8 to Figure 2.12) follow the same trends described above for Ashton Court. In every case, $R_m(\text{DA}) > R_m(\text{SO}) > R_m(\text{RW})$ for both binary and weighted data. In general, distributions of robustness are broader for weighted data than binary data.

2.3.d Effect of manipulating degree distribution

Compared to the results of the binary extinction models for the true AC network, we found that narrowing the degree distributions caused the robustness distribution *f(R)* to be narrower, and this was especially so when the plant degree distribution was manipulated (see Figure 2.5). This confirms that the observed, highly skewed, plant degree distribution of the AC network produces the broad robustness distributions we generate for this network. Note though that median robustness R_m remains in the same order (RW<SO<DA) in every case, showing the consistency of effect from these models.

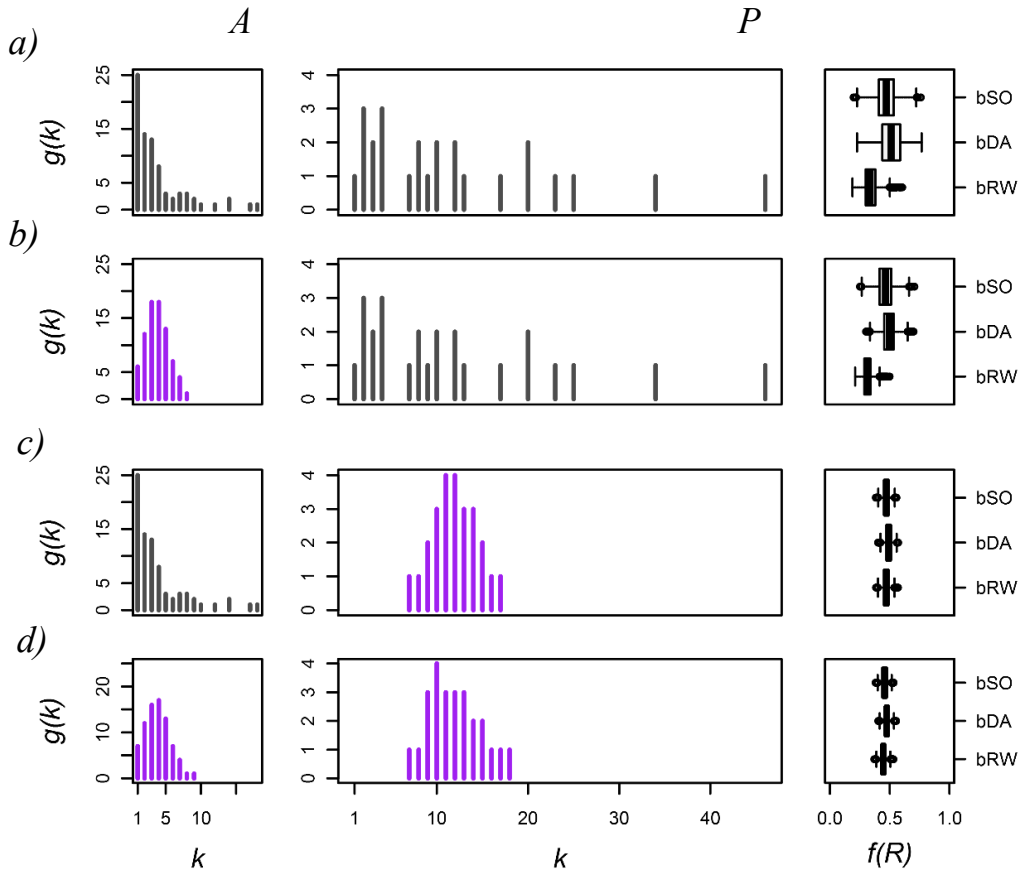


Figure 2.5 The effect of node degree distribution on robustness distribution f(R) for ***a)*** *the binary Ashton Court network and* ***(b-d)*** *manipulated networks as described in section 2.2.h. Left column* ***A)****: pollinator degree distribution (grey - observed; purple – manipulated); central column* ***P)****: plant degree distributions; right column: summaries of f(R) from the bSO, bDA and bRW extinction models. Box-plots, with central lines showing median, boxes showing inter-quartile range, and whiskers showing the 95 % (2.5–97.5 %) interval.*

2.3.e Extinction rank of plant species, and the effect on R

Plant degree is a predictor of the plant's extinction rank in the DA and RW models (Figure 2.6). In the SO models, the rank should be constant for all plant species, irrespective of degree, because the extinction sequence is entirely random. In contrast, the observed extinction ranks of two example plant species from the DA and RW models are clearly skewed (Figure 2.6 (***c***) and (***d***)). In the DA models, median extinction rank (r_m) is positively correlated with plant degree (bDA: $\rho = +0.803$, $p < 0.0001$; wDA: $\rho = +0.420$, $p = 0.03$). For the RW models, r_m is negatively correlated with k (bRW: $\rho = -0.960$, $p < 0.0001$; wRW: $\rho = -0.820$, $p < 0.0001$).

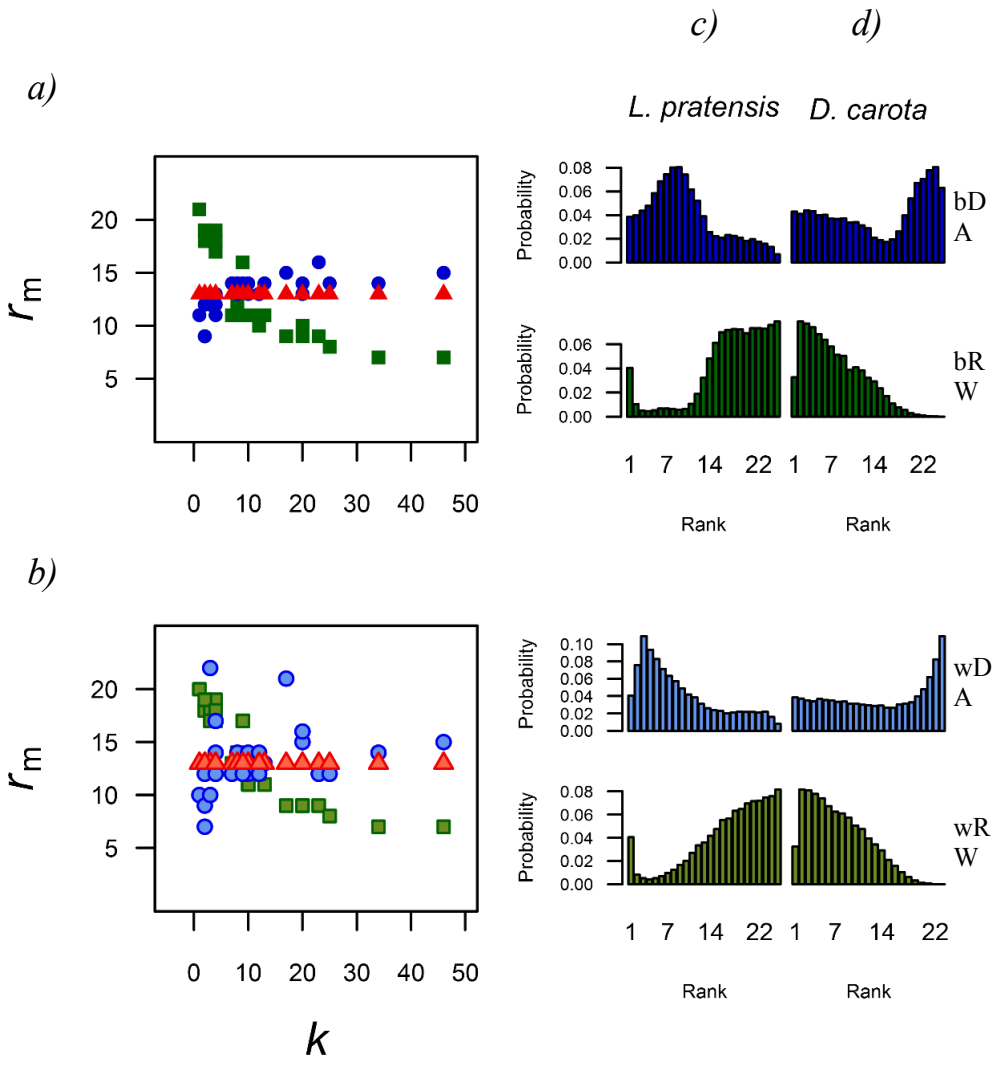


*Figure 2.6 Variation of median extinction rank r_m with degree (k) for all 25 plant species in the Ashton Court network for the three extinction models (SO: red, DA: blue and RW: green) and for **a)** binary and **b)** weighted edges. Spearman's rank correlation show that all these associations are significant: positive for DA (blue) and negative for RW (green). Extinction rank distribution $h(r)$ for two plant species **c)** Lathyrus pratensis (k=2) and **d)** Daucus carota (k=46) produced by the DA (blue) and RW (green) models.*

In other words, for the DA models, well-connected plants are resistant to extinction; the model preferentially prunes the low degree plants so network robustness is high compared to the SO models (Figure 2.4). In contrast, in the RW models plants with high degree are more vulnerable to extinction (the model preferentially "homes in" on well-connected plants) so network robustness is low compared to the SO models (Figure 2.4).

2.4 Discussion

Robustness R is a valuable quantitative metric for describing and comparing the vulnerability of ecological networks to simulated extinctions. We confirm, through our framework of extinction models, that R is a consequence of both the model itself and the network structure. Our analysis reveals the mechanisms and fundamental network properties that drive observed trends in robustness.

Knockout extinction models that calculate robustness have been around for over a decade and the list of ecological rules they employ is growing. Building on the models of Memmott *et al.* (2004), Kaiser-Bunbury *et al.* (2010) and Vieira and Almeida-Neto (2015), we have brought together a suite of directly comparable knockout extinction models and applied them here to plant–pollinator networks. We have used an extinction threshold (pollinators can go extinct before all their plants go extinct and vice versa) that can be applied to all nodes. This addition has an ecological motivation - plants may decline to extinction due to reducing pollination (as modelled by Traveset *et al.,* 2017), and adds greatly to the flexibility of the model. Having $T < 1$ allows us to create weighted versions of our models and provides the potential for feedback between the trophic levels and, hence, avalanches of extinctions cascading across the network (*e.g.*, as shown by Campbell *et al.*, 2012 and Vieira & Almeida-Neto, 2015). Cascades are more likely as T is decreased. We chose a middle value of T (0.5). The exact value chosen is not a vital ingredient of this work but can make a big difference to mean robustness (Figure 2.3). We therefore recommend that researchers test at least the qualitative robustness of their conclusions to varying values of threshold.

All our extinction models, in binary and weighted form, produce a broad distribution of robustness values $f(R)$ for each network that we analysed, indicating that there are aspects of the structure of the network that cause this variation. We found the degree distribution of the plants, in particular, to be an important driver of robustness variation. Plant–pollinator networks tend to have fewer plant species than pollinator species ($P < A$), so the potential for

a skewed plant degree distribution is greater, thus making it more influential on robustness in our test network (Memmott, 1999). Of the six networks we analysed, those that have one particularly highly connected plant (Ashton Court—Figure 2.4, and Hickling—Appendix: Figure 2.11) have the broadest *f(R)*; those with a more homogenous plant degree distribution are narrower. We note in passing that the largest plant degree is strongly correlated with nestedness in these networks (Appendix: Figure 2.13).

Though 'robustness' has in the past been used to suggest priorities for conservation or management (Devoto *et al.*, 2012; Pocock, Evans and Memmott, 2012), extinction models are not an attempt to predict precisely how an ecosystem would collapse. They do, nonetheless, offer a means to quantify and compare the structure of ecological networks, but to do this we need to ensure we are comparing like-for-like.

Plant–pollinator communities are increasingly described with weighted interactions. We found (Figure 2.4) that introducing weighted interactions has the effect of amplifying the outcomes observed for binary data: the inter-quartile range of the robustness distribution *f(R)* increases in all models for weighted networks, and the shifts in median robustness for DA and RW compared to SO are larger. Weights tend to increase the skew of the plant degree distribution because high-degree species accumulate high edge weights and low degree species only gain a small fraction of the overall weight in the network. This exaggerates effects in *f(R)* and highlights the importance of including interaction weights in robustness analysis, and in exploring all of the distribution *f(R)*, not just its central tendency. Future work should continue to explore the full effects of weighted data.

There are different ways in which extinction models can use feedback between trophic levels and we developed two illustrative models: the Deterministic Avalanche (DA) and the Random Walk (RW) models. These models (and others like the cascade model developed by Vieira & Almeida-Neto, 2015) may appear to be generating new outcomes, but in reality, they simply produce a non-random sample of robustness values from those generated by a simple SO model. The AC dataset generated a very wide range of *R* values, all of which can be realized in the SO models. The DA and RW models preferentially sample extinction sequences to produce skewed subsets of the SO outcomes (the *P!* extinction sequences are not all equally likely, and some will be impossible). The DA Model preferentially samples nodes that are 1 step away from each other in the network and extinctions can "ripple out" from each trigger. In some cases, the DA model produces a double-peaked *f(R)* distribution. This corresponds to networks where the highest plant degree, as a fraction of the number of pollinators, is large - the Ashton Court and Hickling networks for example. In contrast to DA, in the RW model plant extinctions tend to jump

from plant to plant away from a trigger. Although both the DA and RW models are ecologically credible, they produce opposing results, demonstrating the influence of the model on the assessment of robustness. It is important for researchers using robustness models to have a clear justification for the model they use, and a clear understanding of how much their results are influenced by the model as well as the network data.

All of these extinction models are designed to be applied to real ecological network data. Therefore, it is vital to consider the quality and reliability of the data being used. Empirical pollination networks vary hugely in sampling method, period of collection and taxonomic resolution, all of which can affect metrics of network structure. Factors such as relative species abundance and time of sampling can lead to over- or underestimating the degree of a plant species in a network (*e.g.,* Blüthgen, Menzel and Blüthgen, 2006).This will affect the outcomes of knock-on extinction models and could easily over- or underestimate the robustness and the importance of particular plant species. We caution against comparing the outcomes of extinction models across multiple networks, for example, in meta-analyses or comparative analyses, without consideration of the data and the methods used to collect them. CaraDonna *et al.* (2017) highlight the potential pitfalls of assuming that a network constructed by aggregating samples over time is an appropriate representation of a community. Further work in understanding temporal variation and the description of fully resolved plant–pollinator networks is key to improving the utility of extinction models.

Current robustness models still lack the biological realism needed to make reliable ecological predictions. They are, however, useful for understanding and separating the effects of mechanism and network structure. We recommend therefore that researchers seeking greater ecological realism in models pay due attention to the details of the models themselves. Ecological conclusions drawn from robustness models may become less surprising when model developments are taken into account. We hope that by improving our understanding of extinction models at a mechanistic level and by setting out different areas of model extension, our work will guide future developments in the analysis of the vulnerability of ecosystems to environmental change.

2.5 Additional points of discussion

This chapter has focused on exploring plant-pollinator community robustness using a theoretical approach. For the rest of this thesis we will continue to explore plant-pollinator

communities but now using observational and experimental methods. Having discussed here the importance of using plant-pollinator network data appropriately for meaningful modelling and taking timescales of observations into account, we will show in the next chapter how the floral resources available in a plant-pollinator community change over time. We will partly address our own suggestion (see above) that understanding temporal variation and the description of fully resolved plant–pollinator networks is key to improving the utility of extinction models.

2.6 Appendix: Supporting Information

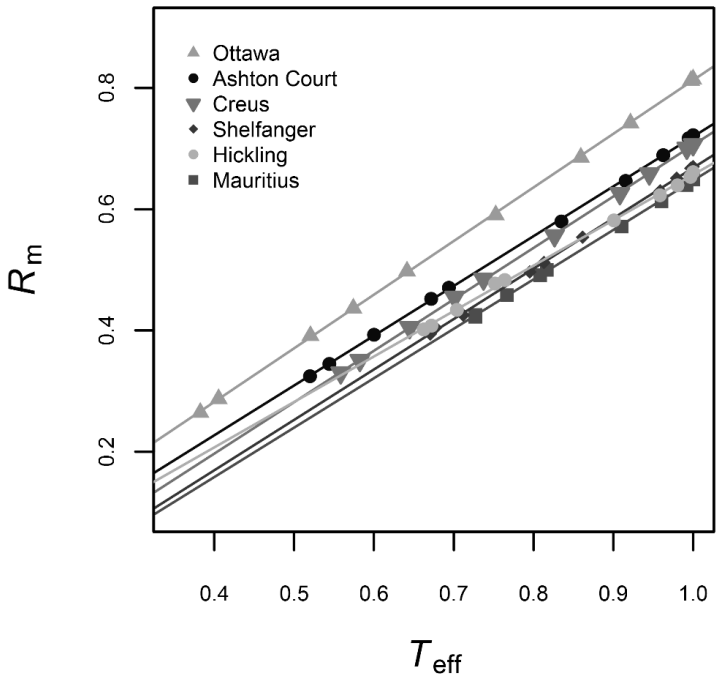


Figure 2.7 The relationship between T_{eff} for the 6 networks (summarised in Table 2.1) used in this chapter. All networks show a linear trend – lines are plotted to guide the eye. All are near parallel with the exception of Hickling which lies very close to Shelfanger and crosses in the middle – the R_m values for Hickling are less representative as the f(R) distributions are so broad.

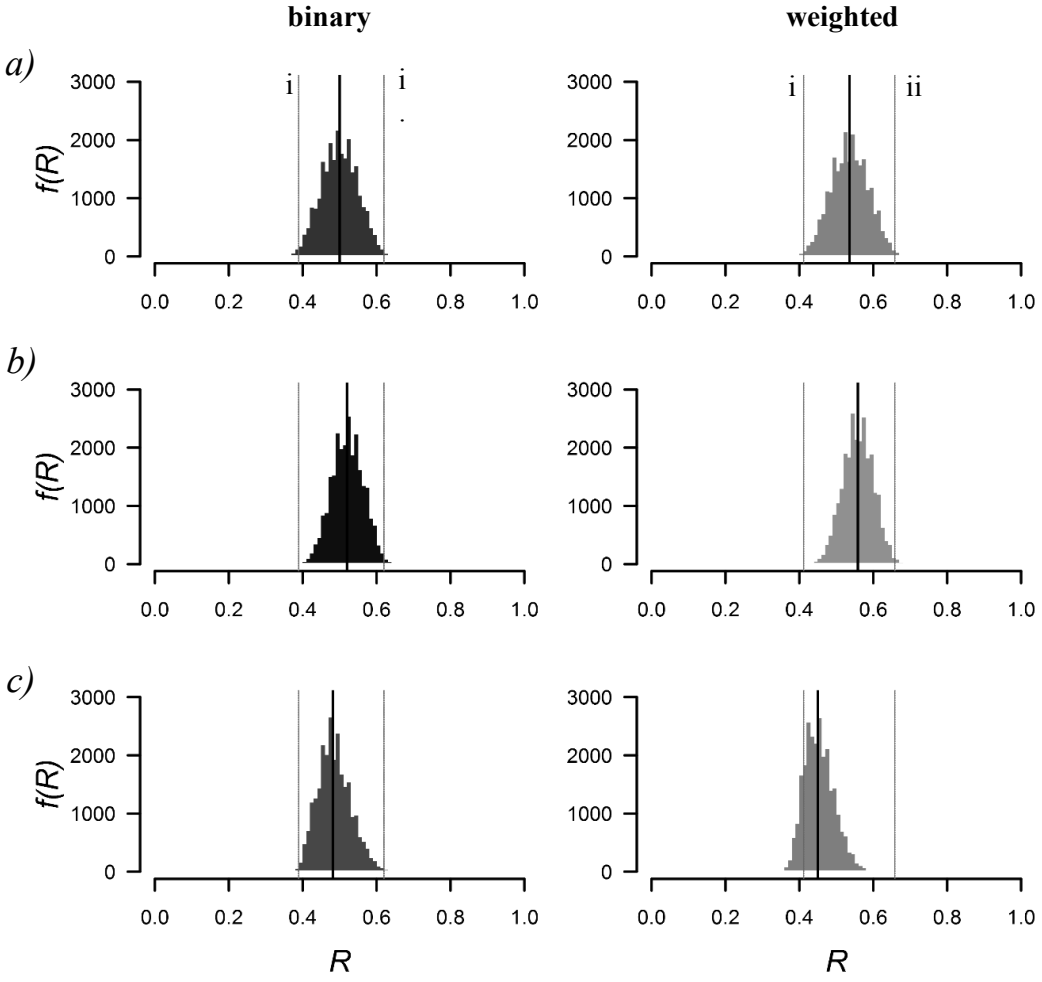


Figure 2.8 The distribution of robustness f(R) for the Ottawa network, binary (left column) and weighted (right column), generated by the 3 extinction models: (a) Secondary Only (SO), (b) Deterministic Avalanche (DA) and (c) Random Walk (RW). Median robustness R_m for each distribution is indicated by the black vertical line. Thin, grey lines indicate R values for the SO model when plants are removed in increasing; (i) b:0.389, w:0.412; and decreasing order (ii); b:0.620, w:0.658. The Ottawa f(R) distributions are not particularly broad. R_m(DA) > Rm(SO) > Rm(RW). The observed breadth and shifts correspond to the homogeneity of the network; the plant degree distribution is less skewed than other plant-pollinator networks. All Ottawa f(R) distributions cross (i) and (ii), particularly so for wRW. This is not surprising as many plant nodes have the same degree and therefore there are many node sequences in degree order – (i) and (ii) are not unique.

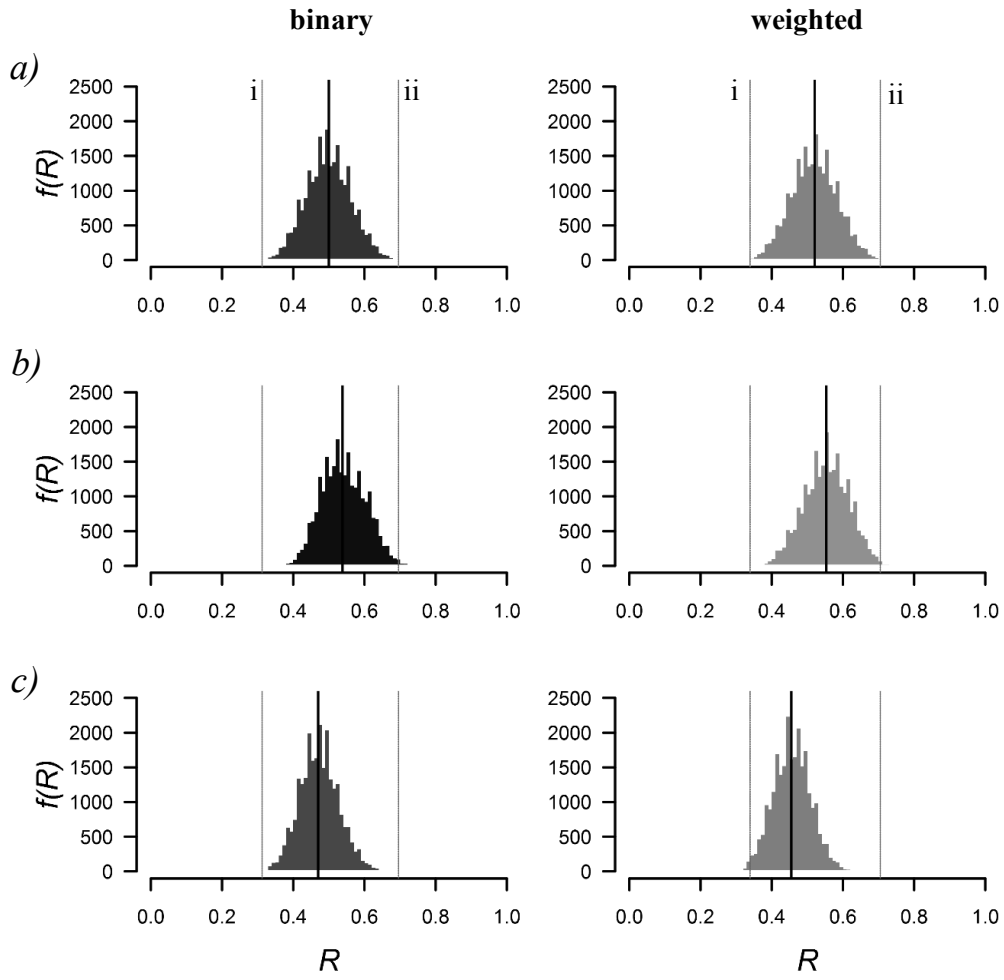


Figure 2.9 The distribution of robustness f(R) for the Mauritius network, binary (left column) and weighted (right column), generated by the 3 extinction models: ***(a)*** *Secondary Only (SO),* ***(b)*** *Deterministic Avalanche (DA) and* ***(c)*** *Random Walk (RW). Median robustness R_m for each distribution is indicated by the black vertical line. Thin, grey lines indicate R values for the SO model when plants are removed in increasing (i); b:0.314, w:0.339; and decreasing order (ii); b:0.696, w:0.705. The Mauritius $f(R)$ distributions are quite broad. R_m(DA) > R_m(SO) > R_m(RW). The observed breadth and size of shifts correspond to the homogeneity of the network.*

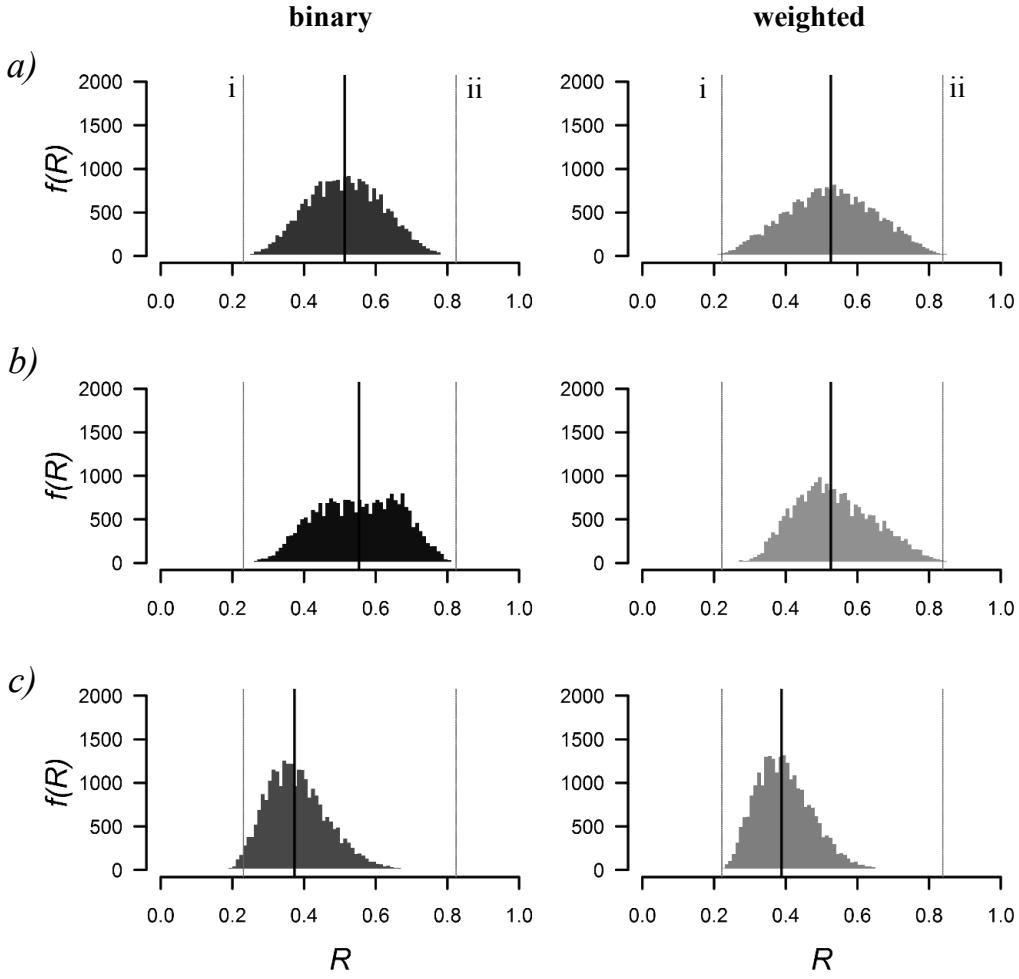


Figure 2.10 The distribution of robustness f(R) for the Shelfanger network, binary (left column) and weighted (right column), generated by the 3 extinction models: ***(a)*** *Secondary Only (SO),* ***(b)*** *Deterministic Avalanche (DA) and* ***(c)*** *Random Walk (RW). Median robustness* R_m *for each distribution is indicated by the black vertical line. Thin, grey lines indicate R values for the SO model when plants are removed in increasing (i); b:0.231, w:0.222; and decreasing order (ii); b:0.825, w:0.839. The Shelfanger* $f(R)$ *distributions are broad.* $R_m(DA) \gtrsim R_m(SO) > R_m(RW)$*. Note the left skewed peak for wDA (though* $R_m(DA) \gtrsim R_m(SO)$*). The breadth and size of shifts correspond to the homogeneity of the network.*

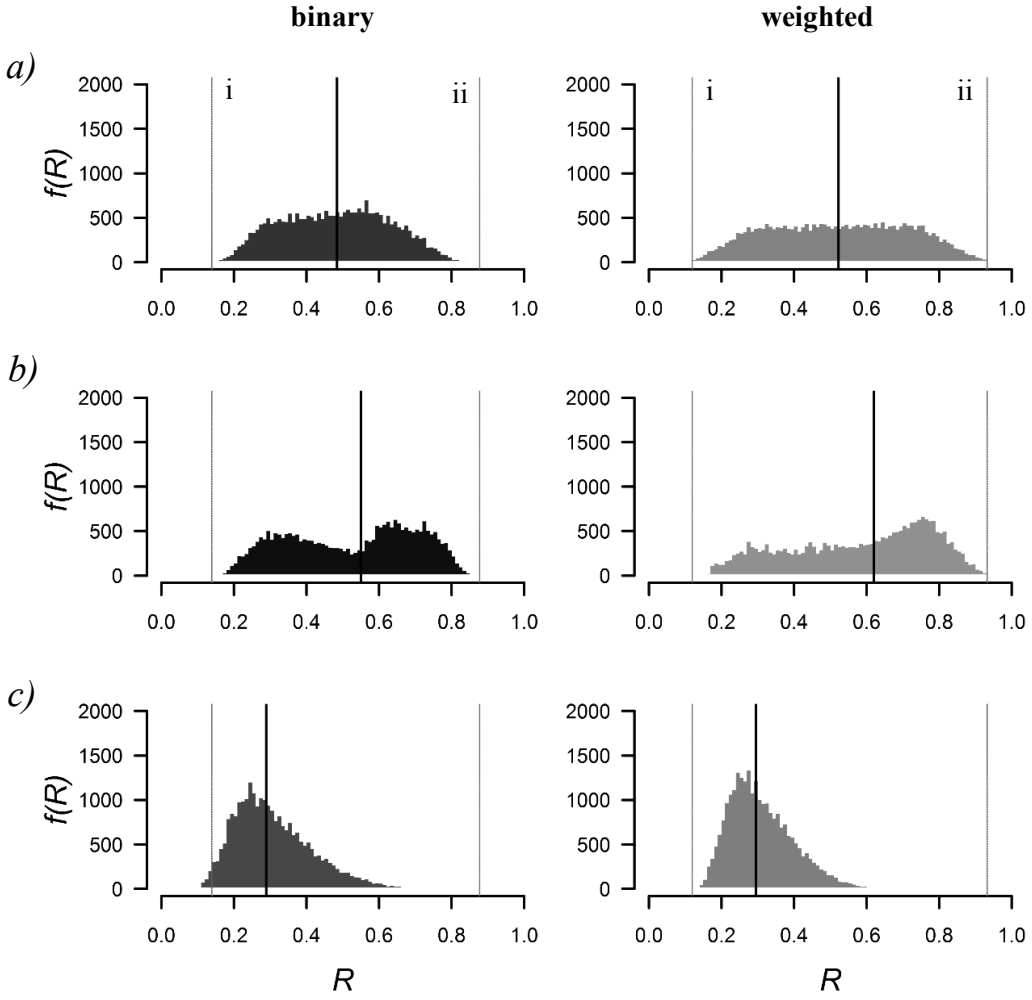


Figure 2.11 The distribution of robustness f(R) for the Hickling network, binary (left column) and weighted (right column), generated by the 3 extinction models: ***(a)*** *Secondary Only (SO),* ***(b)*** *Deterministic Avalanche (DA) and* ***(c)*** *Random Walk (RW). Median robustness R_m for each distribution is indicated by the black vertical line. Thin, grey lines indicate R values for the SO model when plants are removed in increasing (i); b:0.140, w:0.120; and decreasing order (ii); b:0.8775, w:0.9325. The Hickling $f(R)$ distributions are quite broad. $R_m(DA) > R_m(SO) > R_m(RW)$. The very broad distributions, with strong shifts between models correspond to the highly skewed plant degree distribution and network heterogeneity.*

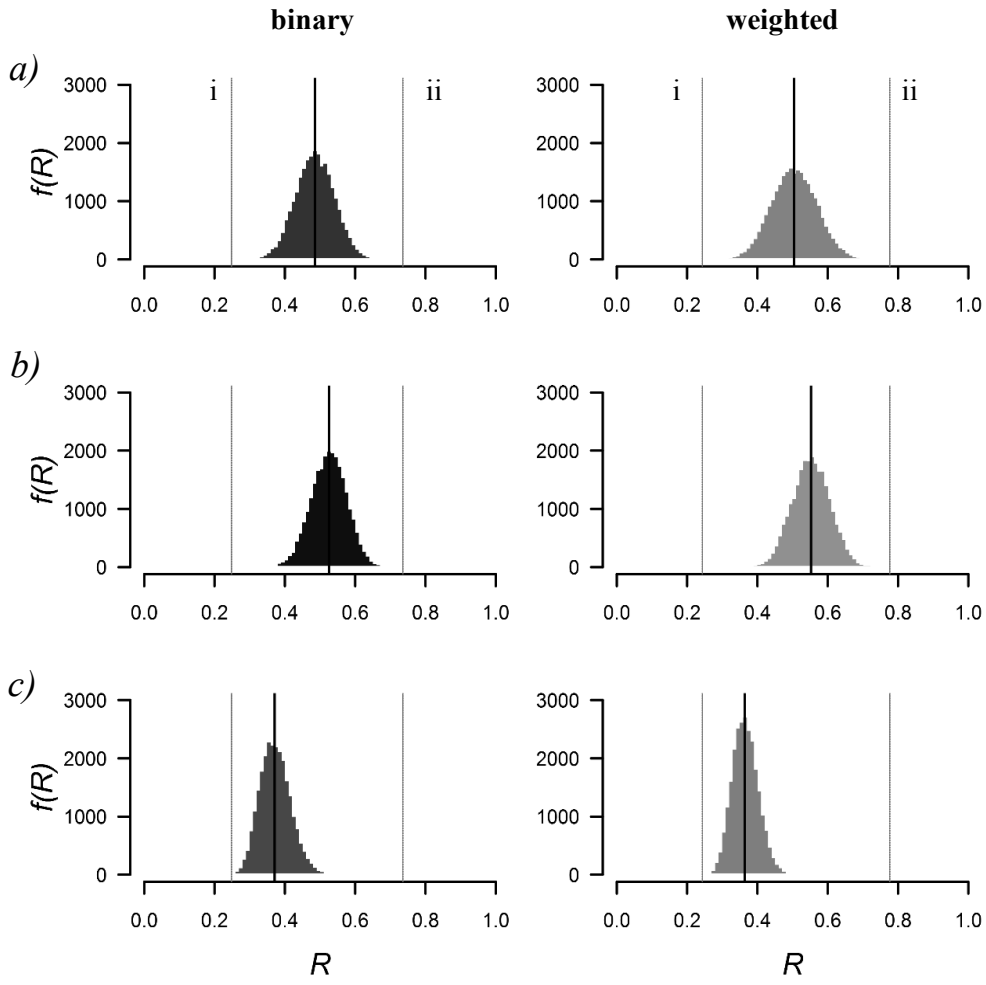


Figure 2.12 The distribution of robustness f(R) for the Creus network, binary (left column) and weighted (right column), generated by the 3 extinction models: ***(a)*** *Secondary Only (SO),* ***(b)*** *Deterministic Avalanche (DA) and* ***(c)*** *Random Walk (RW). Median robustness R_m for each distribution is indicated by the black vertical line. Thin, grey lines indicate R values for the SO model when plants are removed in increasing (i); b:0.249, w:0.243; and decreasing order (ii); b:0.736, w:0.777. The Creus $f(R)$ distributions are quite broad. . $R_m(DA) > R_m(SO) > R_m(RW)$. The observed breadth and shifts correspond to the homogeneity of the network. All Creus $f(R)$ distributions sit well within (i) and (ii), indicating that degree order sequences are unique cases of extreme robustness values and unlikely to occur in 25,000 simulations.*

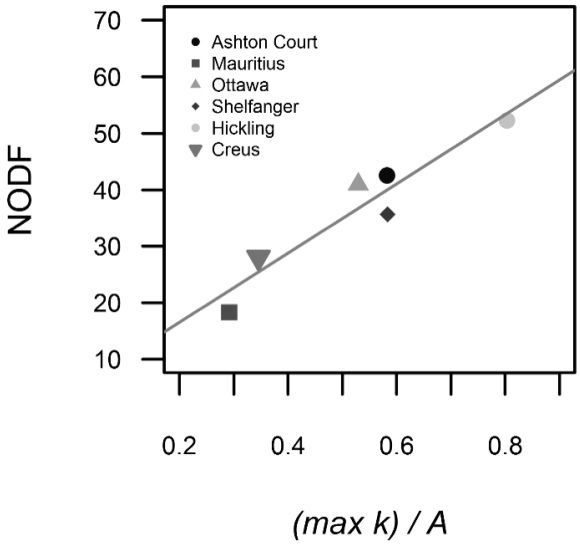


Figure 2.13 Correlation between the normalised, largest plant degree (max k)/A, and nestedness (NODF) (Almedia-Neto et al. 2008). Spearman's rank correlation: $\rho = +0.83$, $p = 0.04$. Line plotted to guide the eye.

Chapter 3

Phenology and turnover of flowering plants and bumblebee species

Abstract

Plant-pollinator communities change over time; the species present change through daily, seasonal and yearly succession, and the interactions between species change in response to the dynamics of the community and environmental stimuli. However, plant-pollinator network data is often treated as static or lumped over long time periods, leading to a loss of temporal resolution. In order to understand this mismatch between 'real-world' dynamics and static of plant-pollinator network data, we explored how a plant-pollinator community changed over time in terms of the when plant species were flowering (flowering phenology) and when bumblebees were active and foraging (bumblebee phenology). We collected observations of flowering and bumblebee phenology over spring and summer (a pollination season) in 2016 and 2017 along a species rich transect. We found that:

a) the amount of foraging resource at the site was not consistent between the two years; at peak flowering, plant species had on average 55-58 floral units (FU) in 2017 for every 100 FU in 2016. However, there was significant consistency in plant species progression between the two years; species flowered in the same order. In addition, at any given point the site was dominated by one or two of a small number of plant species (4-5) that were highly abundant.
b) that bumblebee phenology was significantly longer than flowering phenology, indicating that bumblebee species would have to interact with different plant species over time. On average, bumblebees were first observed feeding on a plant species 18 days after first flowering, and 18 days before it reached peak flowering.
c) that the majority of flowering phenologies were positively skewed, meaning that bumblebees (and other pollinators) are likely to be switching from a resource that is rapidly declining to one that is more gradually increasing.

3.1 Introduction

Plant-pollinator communities are dynamic; as we would expect with any ecological community, they change over time. In temperate regions, most plant-pollinator communities are active throughout the spring and summer. During this 'pollination season' there will be changes in which species are present, abundant and interacting. Therefore, plant-pollinator network data that is sampled from a real plant-pollinator community will be influenced by the timing and period of sampling. Despite this, plant-pollinator network data is often treated as static, or considered in terms of relatively simple 're-wiring' dynamics (as summarised in Poisot, Stouffer and Gravel, 2015). As we demonstrated in Chapter 2, observational plant-pollinator network data can serve as the basis for theoretical modelling of the stability of plant-pollinator communities over ecological time. This kind of modelling is undoubtedly valuable in expanding our understanding of plant-pollinator communities. However, it is equally important to understand how more short-term plant-pollinator community dynamics impact sampled plant-pollinator network data in order to make the most of theoretical approaches and understand the robustness of plant-pollinator communities in real time.

Community dynamics through space and time are well studied (Czaran and Bartha, 1992). In the context of ecological networks, and plant-pollinator communities in particular, there are three key dynamics to consider; the turnover of plant species, the turnover of pollinator species and the turnover of interactions (Poisot *et al.*, 2012). One way to quantify species turnover is to use a measure of beta-diversity based on species richness (how many species are present). One of the earliest measures of beta-diversity, proposed by Whittaker (1960) calculates how much the species richness (number of different species present) of a whole region differs from the average species richness in a sample location within that region:

$$\beta = \frac{\gamma}{\alpha}$$

where $\gamma =$ species richness at the regional level, and $\alpha =$ average species richness of a sample location. This can be adapted to measure species turnover between two samples over space or time, amongst many possible variations and applications (reviewed in Koleff, Gaston and Lennon, 2003). In this chapter, we use beta diversity to measure the turnover of plants and pollinators over time. Plants and pollinators must be present at the same time in order to be able to interact, though presence alone does not mean that an interaction will occur (Poisot, Stouffer and Gravel, 2015). Interaction turnover can also be quantified using

beta-diversity measures. Poisot *et al.* developed a method to quantify beta-diversity in terms of different components corresponding to the species and interaction turnover (Poisot *et al.*, 2012). We adopt this method in Chapter 4 but highlight it here as it is a useful tool in understanding plant-pollinator network dynamics.

Beta-diversity and other measures of community change have been used to study plant-pollinator networks to great effect. Kaiser-Bunbury *et al.* (2014) collected plant-pollinator network data from 6 sites, every month, for 8 months. They modelled various community processes including turnover. They found that 69% of the variation in plant-pollinator interactions could be explained by community composition (turnover and abundance of plant and pollinator species) and that ~45% of interaction patterns could be explained by changes in the floral resource (plant species presence and abundance). Similarly, CaraDonna *et al.* (2017) collected plant-pollinator network data weekly through the pollination season of a sub-alpine community over three years. They found that weekly turnover was high and mainly driven by interaction turnover. There was consistent seasonal progression of species, suggesting that species turnover is predictable to some extent across seasons.

Taking a scaled-down approach compared to the studies above, we wanted to examine and quantify how much a plant-pollinator community changed over a pollination season at our specific field site. We wanted to understand the implications of community dynamics on plant-pollinator network data, and to describe the community at our specific field site so that we could plan the field experiment presented in Chapter 4. To do this, we collected records of flowering phenology, bumblebee phenology and plant-bumblebee interactions from March to September in 2016 and 2017.

Flowering phenology can be defined as the timing of the period when a flower has open petals – when it is 'flowering'. There are various similar measures of flowering in the literature. We adopt that of Last, (2001); when the 'stamens or stigmas can be seen without pushing petals aside' as shown in Figure 3.1 though note that this does not apply to all plant species so must be assessed using common sense. Flowering phenology is one of the most common types of phenological data collected. Historical datasets (of which there are many examples, e.g. (Margary, 2007) often record only the 'first flowering day' (FFD): the first day that a plant species was observed flowering in a year, within a given population. Here we use a more informative measure; flowering period. Flowering period (or flowering spread) refers to the number of days between FFD and the last flowering day (LFD) of individuals in a given species' population (Sunley, Atkinson and Jones, 2006; Elzinga *et al.*, 2007). In some cases, as in this chapter, the number of individual flowers at given times

within the flowering season is also recorded. The resulting flowering phenology tells us when a given plant species was flowering, for how long, and in what numbers. Crucially, this tells us when a plant species can be visited by pollinator species.

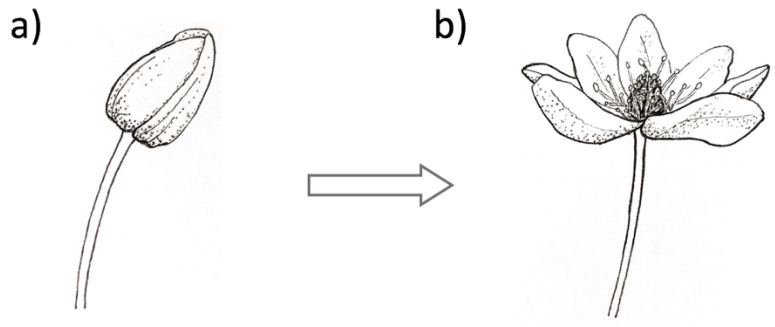


*Figure 3.1 An illustration of Anemone nemorosa (Wood anemone) showing **a)** Before flowering when only a bud is visible and **b)** flowering, when the petals are open with the stamens and stigmas (in the centre of the flower) accessible.*

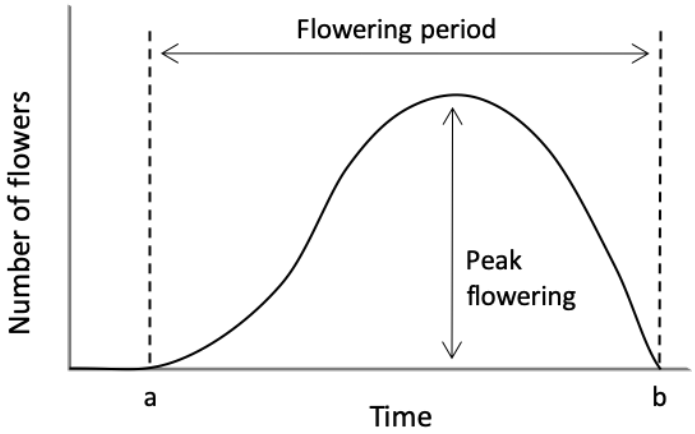


Figure 3.2 A schematic of a simplified flowering phenology showing the flowering of a plant species over time. The first flowering day (FFD) is indicated by the dashed line at 'a' and the last flowering day (LFD) is indicated by the dashed line at 'b'. In many cases the rise and fall of flowering peak is not symmetrical, as indicated in this schematic. We have shown a flowering phenology with a single peak, but it is possible for a plant species to have multiple flowering peaks.

The second type of phenological data examined in this chapter is bumblebee phenology. Like other social insects, bumblebees have a colony level life cycle (Prys-Jones and Corbet, 2011) – see Chapter 1, section 1.4 for a detailed explanation. We use the term bumblebee phenology to refer to the period when bumblebees of a given population are

foraging for floral resources. This period spans from when the queen emerges from hibernation and establishes a nest (in early Spring) until the point when the workers and males die, and new queens enter hibernation (late Summer). We measured bumblebee phenology using observations of foraging bumblebee individuals. We chose to use bumblebees as our example pollinator because they are easy to spot and identify (to morphotype) in the field and their colony life cycle is well understood.

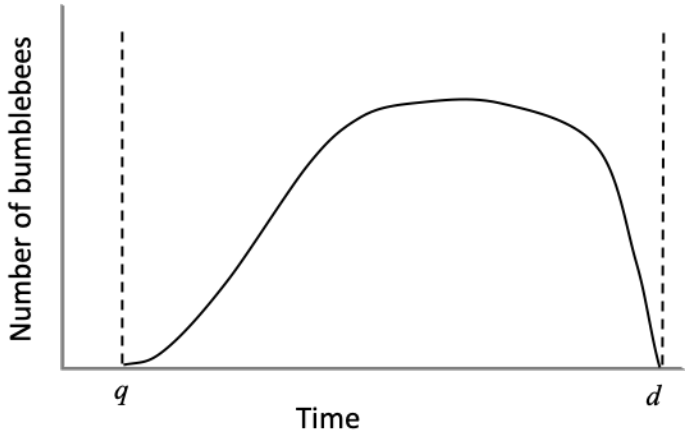


Figure 3.3 A schematic of a simplified bumblebee phenology showing the number of bumblebees observed foraging over time. The phenology spans the period from the emergence of the queen (indicated by the dashed line at q) to the death of the colony and hibernation of new queens (indicated by the dashed line at d). In our observations we focus on the overall population patterns that are a combination of multiple colony phenologies at the given site.

In this chapter we present phenological records for plant and bumblebee species collected from March to September in 2016 and 2017. We recorded the numbers of flowering plant species, and observations of plant-bumblebee interactions along a ~700m fixed transect that passed through three different habitats. In addition, we recorded bumblebee individuals observed foraging along the transect (and what they were foraging on), resulting in an approximation of the phenology for bumblebee species foraging at the site. Our main objective was to collect evidence for the variation in the flowering phenology of plant species at our field site in order to:

i) demonstrate that floral resources change over time and therefore pollinator species must react to changes in their foraging environment,

ii) show that the timing of sampling can greatly affect the species observed, and therefore the interactions, in a plant-pollinator network,

iii) explore how bumblebees (our example pollinators) interacted with a changing floral environment over time,

iv) trial field methods and plan the experiment in Chapter 4 with detailed knowledge of the site, and inform the ideas explored in Chapter 5.

It is common knowledge that plant species flower at different times. A given plant community changes as different plant species come into and fall out of flowering, influenced by biological and environmental factors (Rathcke and Lacey, 1985). Our aim was to show exactly how the floral resources available to pollinators change as the phenologies of different plant species overlapped throughout the season on our particular site. First, we present an analysis of the variation in flowering phenology both temporally (throughout the flowering season and across two consecutive years) and spatially (across the three habitats linked by the transect). We then compare bumblebee and flowering phenologies in terms of overlap and flowering phenology skewness and discuss the implications of overlap and skew in terms of interaction turnover and the foraging behaviour of bumblebees.

3.2 Field methods

3.2.a The survey site

The survey took place at a site on Claverton Down, Somerset, UK, (51.382959°N, -2.32148°W). Claverton Down is a hill (204m at its peak) to the east of the city of Bath (Figure 3.4). We surveyed along a transect (approximately 700m) that passed through three adjacent, but distinct habitats. We refer to each habitat as a section of the transect: T1, T2 and T3 (Figure 3.5). The first habitat (T1 ~280m) was the edge of the rough grass and public footpath in the southeast corner of the Bath Golf Club golf course (Golf Course Road, Bath, BA2 6JG). The rough is a mix of tall grasses sown with wild flower species and the footpath is mainly bordered by trees and bramble. The second habitat (T2 ~90m) was part of Bathampton wood, a deciduous woodland on the north east slope of Claverton Down. The third habitat (T3 ~330m) was the flower-rich, northern boundary of Bushey Norwood, a calcareous parkland meadow which is managed by the National Trust. T1 and T3 are nearly parallel, approximately 30m apart and separated by a low, drystone wall, a line of mixed, deciduous tree species and some shrubs and bramble. Previous 'Bioblitz' surveys had taken place on T3 but other than this the transect had not be studied before. Photographs of the three sections of the transect can be seen in Figure 3.6.

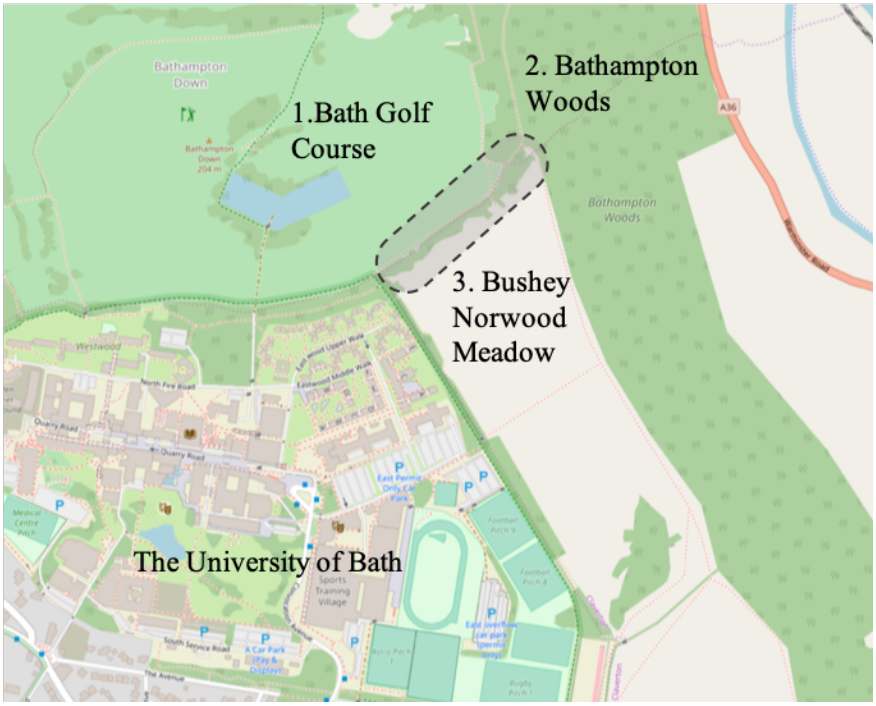


Figure 3.4 A map showing the general location of the transect on Claverton Down, next to the University of Bath (© OpenStreetMap contributors, openstreetmap.org accessed 09.03.18). The transect lies within the area enclosed by the grey dashed line.

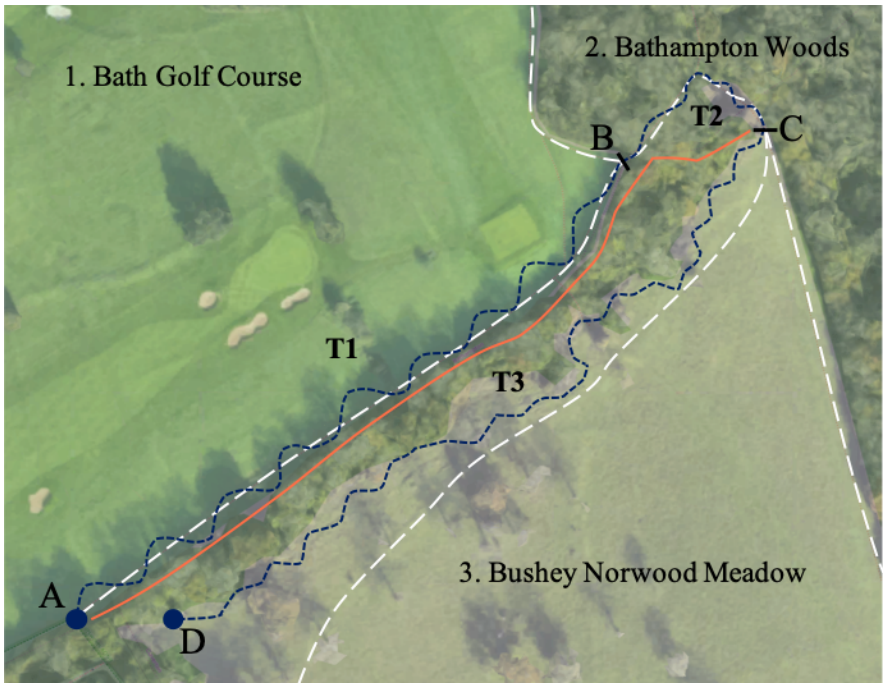


Figure 3.5 An aerial view of the transect (dark, dashed line A-D). The white dashed line indicates the public footpath. The solid red line indicates the location of the wall boundary. A to B is section T1 of the transect. B to C is section T2 of the transect and C to D is section T3 of the transect. A straight measurement from A to D (not following the wiggle of the transect) is approximately 700m long. (Image composite from © OpenStreetMap contributors, openstreetmap.org accessed 09.04.18 and Imagery ©2018 Google, Map data ©2018 Google, accessed 09.04.18

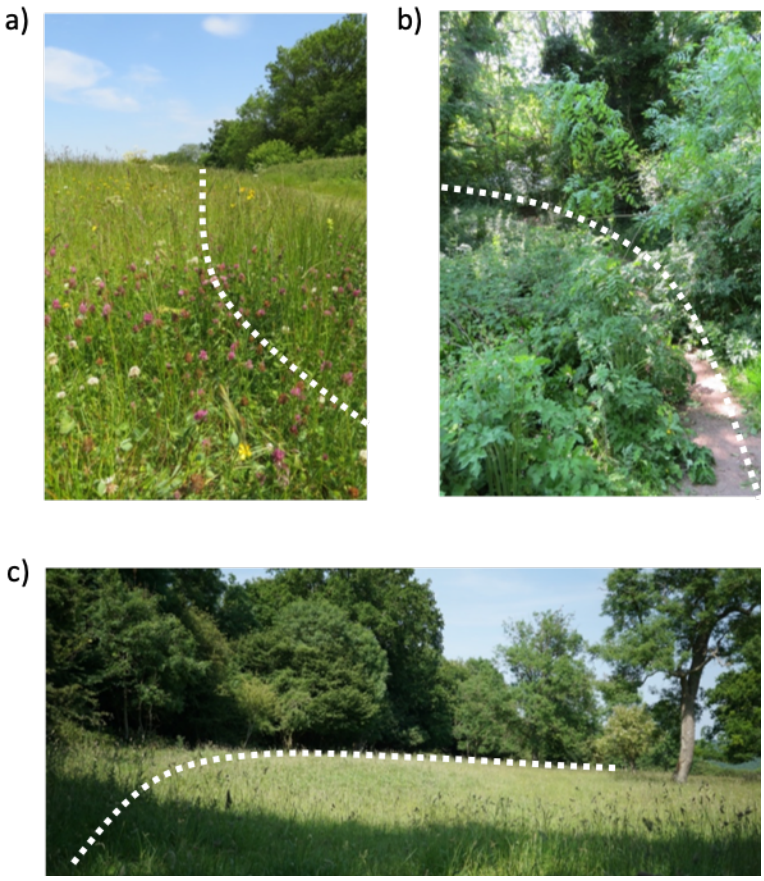


Figure 3.6 Photos of three sections of the transect: ***a)*** *T1, the golf course rough and footpath,* ***b)*** *T2, the woodland and* ***c)*** *T3, the flower-rich grassland meadow. White dotted lines roughly indicate the direction of the phenology transect. The photos of T1 and T2 were taken on the 15th of June in 2016, the photo of T3 was taken on the 21st of June in 2017.*

3.2.b Survey methods

We surveyed along a fixed transect, through T1, T2 and T3 as depicted in Figure 3.5. The path of the transect was specifically chosen to cover the areas with the highest density of flowering plant species (determined from pre-survey examination of the area). We aimed to survey regularly, at least once a week, and when the weather conditions were best (sunny and warm) according to a local forecast. Miranda Bane walked the transect roughly twice a week from the 17th March to the 22nd of September in 2016. Based on the comparatively low abundance of flowering species and bumblebees recorded in the woodland habitat (T2), it was decided that that this section would not be surveyed in 2017. Sections T1 and T3 were surveyed from the 25th February to the 21st September in 2017. During each survey the numbers of flowering plant species, and the interactions between bumblebee individuals and flowering plant species, within 1m either side of the transect were recorded.

Plants were identified to species level in almost all cases. A small number of morphologically similar species were lumped for efficient surveying. These are summarised in Table 3.1. The numbers of flowering plant species along the transect were recorded in floral units (FU), defined by us for the purposes of counting. In some species, this is simple. For example, 1 floral unit of *Primula vulgaris* (primrose) is one flower. However, other species can be harder to count such as *Anthriscus sylvestris* (cow parsley) which has an umbel made up of many tiny flowers, and *Ajuga reptans* (bugle) has a spike made up of several small flowers. In general, we defined flowers as separate floral units if an insect (such as a bumblebee) would have to fly between them. We described different types of floral unit based on five simple morphologies (Figure 3.7). Our definitions of floral units for the plant species recorded at the site are summarised in Table 3.2. Where possible when surveying, each floral unit was counted individually. However, some species were too numerous to count each floral unit and so the number of floral units was estimated by counting the number of floral units in a small patch and multiplying up to the size of the area covered. Whilst walking the transect we also recorded any bumblebee individual within 1m of either side of the transect and the flower species it was foraging on when first observed. Bumblebee species were identified by sight whilst foraging. Occasionally, individuals were caught for closer inspection, then released. Two species common in the south of the UK, *Bombus terrestris* and *Bombus lucorum* workers cannot be separated without DNA analysis and so were grouped as *Bombus lucorum/terrestris* as in other studies (see for example Goulson *et al.* 1998). We aimed to evenly distribute surveying effort along the transect and to spend approximately the same amount of time on each survey (~40mins) to avoid over-sampling bumblebees in flower rich areas.

Table 3.1 Summary of the plant species that were lumped for efficient surveying.

Lumping	**Species**
Cerastium spp.	*Cerastium fontanum*
	Cerastium glomeratum
Crepis spp.	*Crepis biennis*
	Crepis capillaris
Ranunculus spp.	*Ranunculus acris*
	Ranunculus bulbosus
	Ranunculus repens

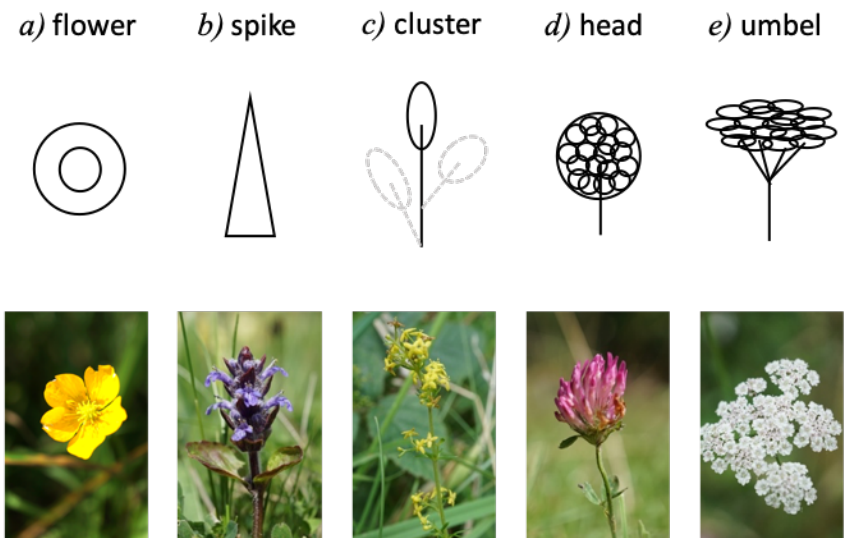


Figure 3.7 Schematic showing the five different types of floral unit classification with examples. Each type is equal to one floral unit: ***a)*** *a single flower,* ***b)*** *a spike,* ***c)*** *a cluster* ***d)*** *a head and* ***e)*** *an umbel. In some cases, for example Bellis perennis (daisy) a single flower (a) is made up of a dense head of tiny flowers surrounded but petals/bracts. This is called a capitulum and in our survey the capitulum is equal to one flower.*

Table 3.2 Floral unit definition of all of the plant species recorded along the transect in 2016 and 2017. See Figure 3.7 for a summary of floral unit categories.

Species	**Common name**	**Floral unit**
Adoxa moschatellina	Townhall clock	cluster
Alliaria petiolata	Garlic mustard	cluster
Capsella bursa-pastoris	Shepherd's purse	cluster
Cardamine hirsuta	Hairy bittercress	cluster
Circaea lutetiana	Enchanters nightshade	cluster
Crataegus monogyna	Common hawthorn	cluster
Galium aparine	Cleavers	cluster
Galium verum	Lady's bedstraw	cluster
Jacobaea vulgaris	Ragwort	cluster
Lamium album	White dead nettle	cluster
Lamium galeobdolon	Yellow archangel	cluster
Lotus corniculatus	Birds-foot trefoil	cluster
Myosotis sylvatica	Wood forget-me-not	cluster
Anemone nemorosa	Wood anemone	flower
Bellis perennis	Daisy	flower (capitulum)
Cardamine pratensis	Cuckoo flower	flower

Cerastium spp.	Mouse-ear species	flower
Crepis capillaris	Smooth hawksbeard	flower (capitulum)
Crepis spp.	Hawksbeard species	flower (capitulum)
Ficaria verna	Lesser celendine	flower
Geranium robertanium	Herb robert	flower
Geum urbanum	Wood avens	flower
Hyacinthoides non-scripta	Bluebell	flower
Hypochaeris radicata	Cat's ear	flower (capitulum)
Leontodon hispidus	Rough hawkbit	flower (capitulum)
Malva moschata	Musk mallow	flower
Narcissus pseudonarcissus	Wild daffodil	flower
Potentilla reptans	Creeping cinquefoil	flower
Potentilla sterillis	Barren strawberry	flower
Primula vulgaris	Primrose	flower
Ranunculus bulbosus	Bulbous buttercup	flower
Ranunculus spp.	Buttercup species	flower
Rosa arvensis	Field rose	flower
Rosa canina	Dog rose	flower
Rubus fruticosus agg.	Bramble	flower
Silene dioica	Red campion	flower
Sonchus arvensis	Perennial sow-thistle	flower (capitulum)
Taraxacum officinale	Dandelion	flower (capitulum)
Tragopogon pratensis agg.	Goats beard	flower (capitulum)
Veronica chamaedrys	Germander speedwell	flower
Veronica filiformis	Slender speedwell	flower
Veronica montana	Wood speedwell	flower
Viola reichenbachiana	Early dog violet	flower
Arctium lappa	Greater burdock	head (capitulum)
Centaurea nigra	Common knapweed	head (capitulum)
Cirsium arvense	Creeping thistle	head (capitulum)
Cirsium eriophorum	Woolly thistle	head (capitulum)
Cirsium palustre	Marsh thistle	head (capitulum)
Cirsium vulgare	Spear thistle	head (capitulum)
Knautia arvensis	Field scabious	head (capitulum)
Medicago lupulina	Black medic	head
Trifolium pratense	Red clover	head
Trifolium repens	White clover	head
Agrimonia eupatoria	Common agrimony	spike
Anacamptis pyramidalis	Pyramidal orchid	spike

Dactylorhiza fuchsii	Common spotted orchid	spike
Glechoma hederacea	Ground ivy	spike
Hypericum hirsutum	Hairy St. John's wort	spike
Plantago lanceolata	Ribwort plantain	spike
Prunella vulgaris	Self-heal	spike
Rhinanthus minor	Yellow rattle	spike
Stachys sylvatica	Hedge woundwort	spike
Vicia cracca	Tufted vetch	spike
Vicia sativa	Common vetch	spike
Anthriscus sylvestris	Cow parsley	umbel
Conopodium majus	Pignut	umbel
Daucus carota	Wild carrot	umbel
Heracleum sphondylium	Hogweed	umbel
Torilis japonica	Upright hedge parsley	umbel

3.3 Overview of the data

In 2016, the phenology transect was surveyed 41 times from the 17th of March to the 22nd of September (roughly twice a week) and in 2017, the phenology transect was surveyed 28 times from the 25th of February to the 21st of September (roughly once a week). Survey days are summarised in Figure 3.8 The numbers of plant species, floral units, bumblebee species and bumblebee individuals recorded are summarised in Table 3.3.

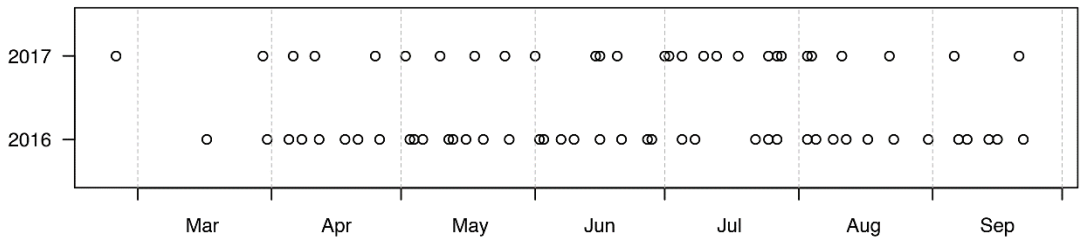


Figure 3.8 The days surveyed in 2016 and 2017. In 2016, the transect was surveyed 41 times. In 2017 the transect was surveyed 28 times.

Table 3.3 A summary of the records from the 2016 and 2017 phenology transect surveys. For the rest of this chapter: (*) lumped plant species (see Table 3.1) are referred to as a single species, (†) the grouped Bombus lucorum and Bombus terrestris are referred to as one species.

	2016	**2017**
Survey days	41	28
Plant species	66 *	56 *
Total floral units	188,281	86,105
Mean floral units per day	4592	3075
Bumblebee species	9 †	7 †
Total bumblebee individuals	593	200

3.3.a Flowering phenology

Figure 3.9 to Figure 3.13 show the flowering phenology data collected from each section of the transect in 2016 and 2017. In each figure, the plant species observed on the given section of the transect, between February/March and September of the given year are organised in order of progression, based on first record, to show how the floral resources change over time.

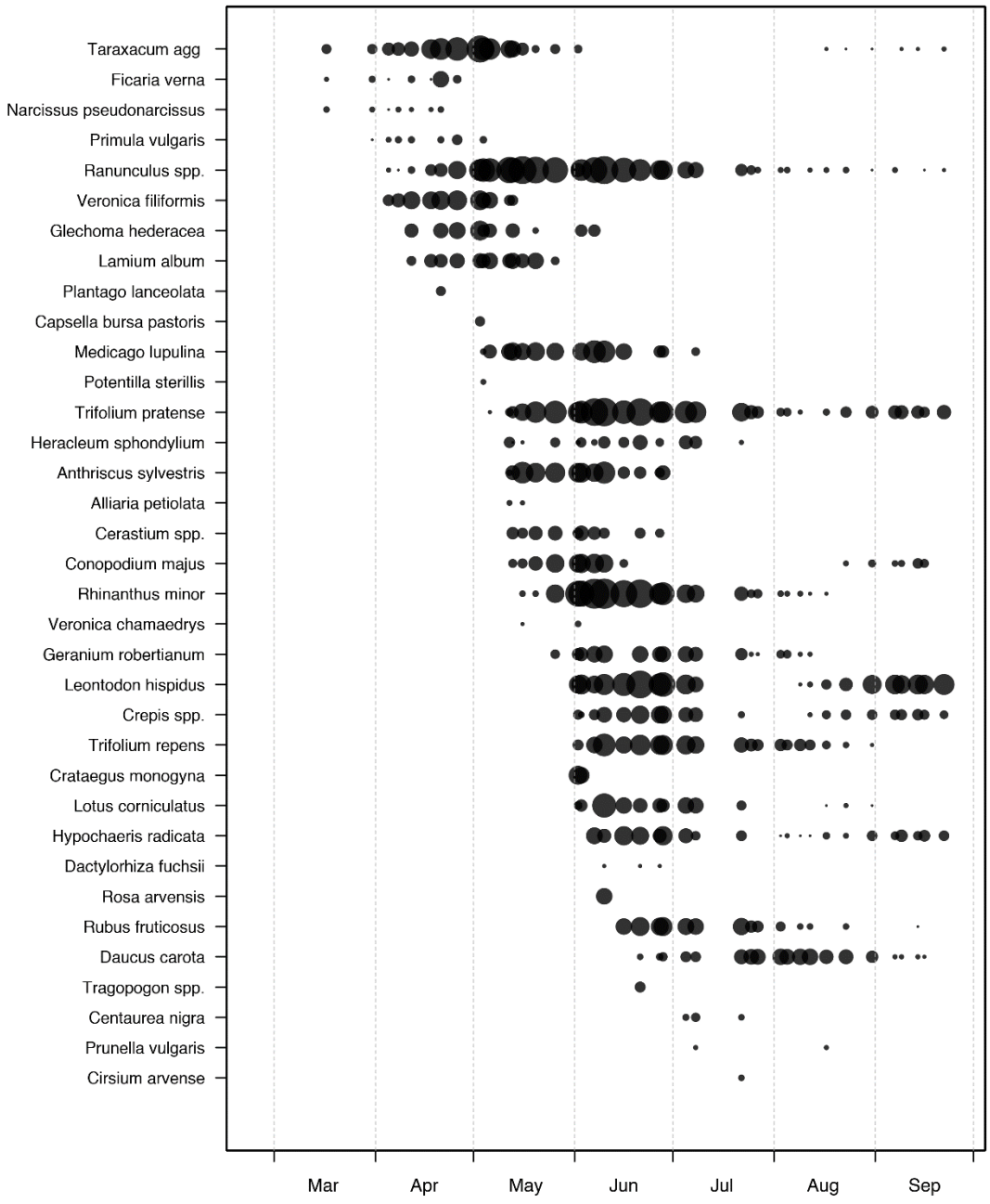


Figure 3.9 Flowering phenologies from T1 in 2016 showing progression of flowering species. In this and the following 4 figures (3.10-3.13): each point indicates when a plant species (listed on the y-axis) was observed flowering. The radius of each point is scaled to the number of floral units observed (log_{10}(floral units + 1)) – consistent across all plots of this kind for plant species.

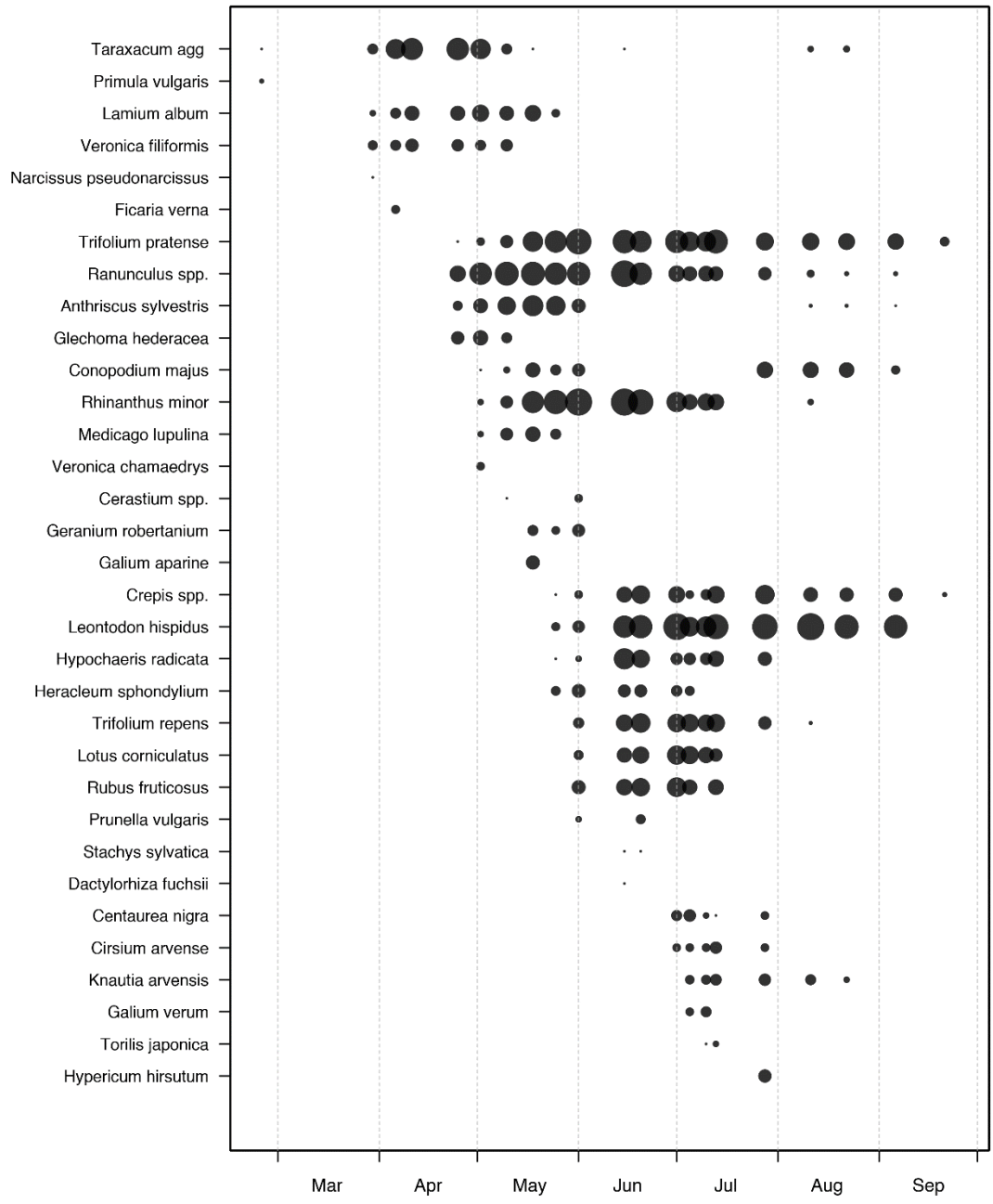


Figure 3.10 Flowering phenologies from T1 in 2017 showing progression of flowering species.

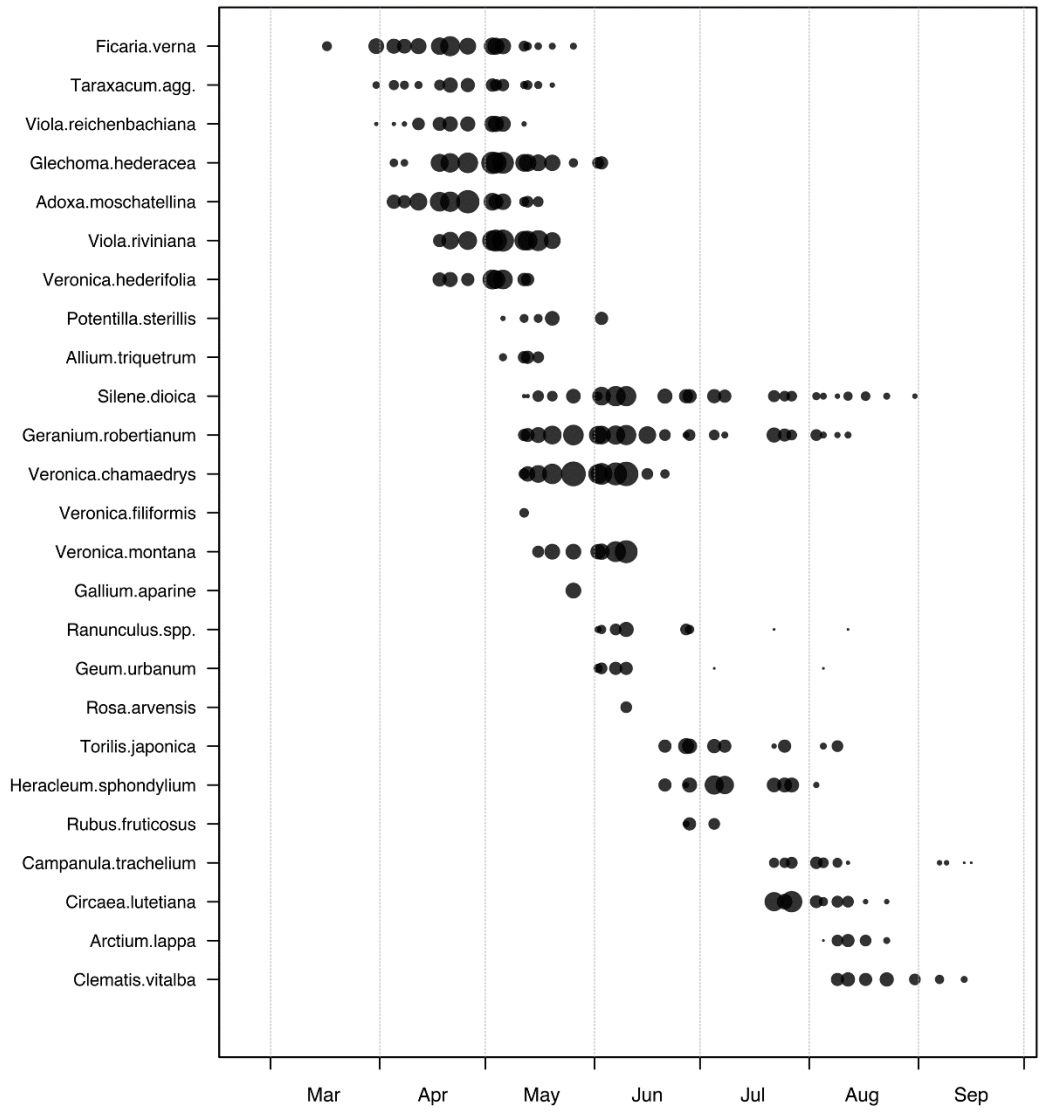


Figure 3.11 Flowering phenologies from T2 in 2016 showing progression of flowering species.

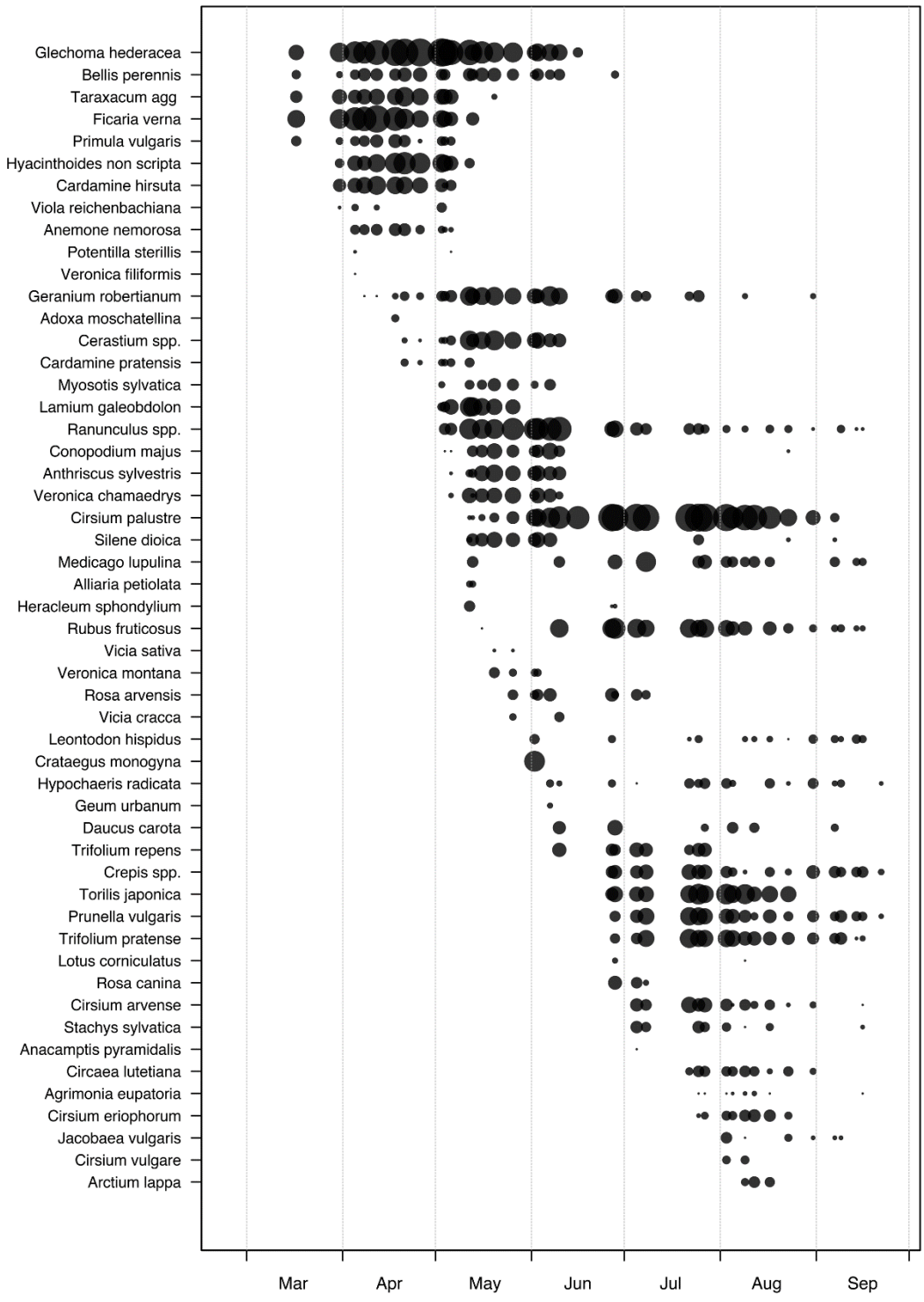


Figure 3.12 Flowering phenologies from T3 in 2016 showing progression of flowering species.

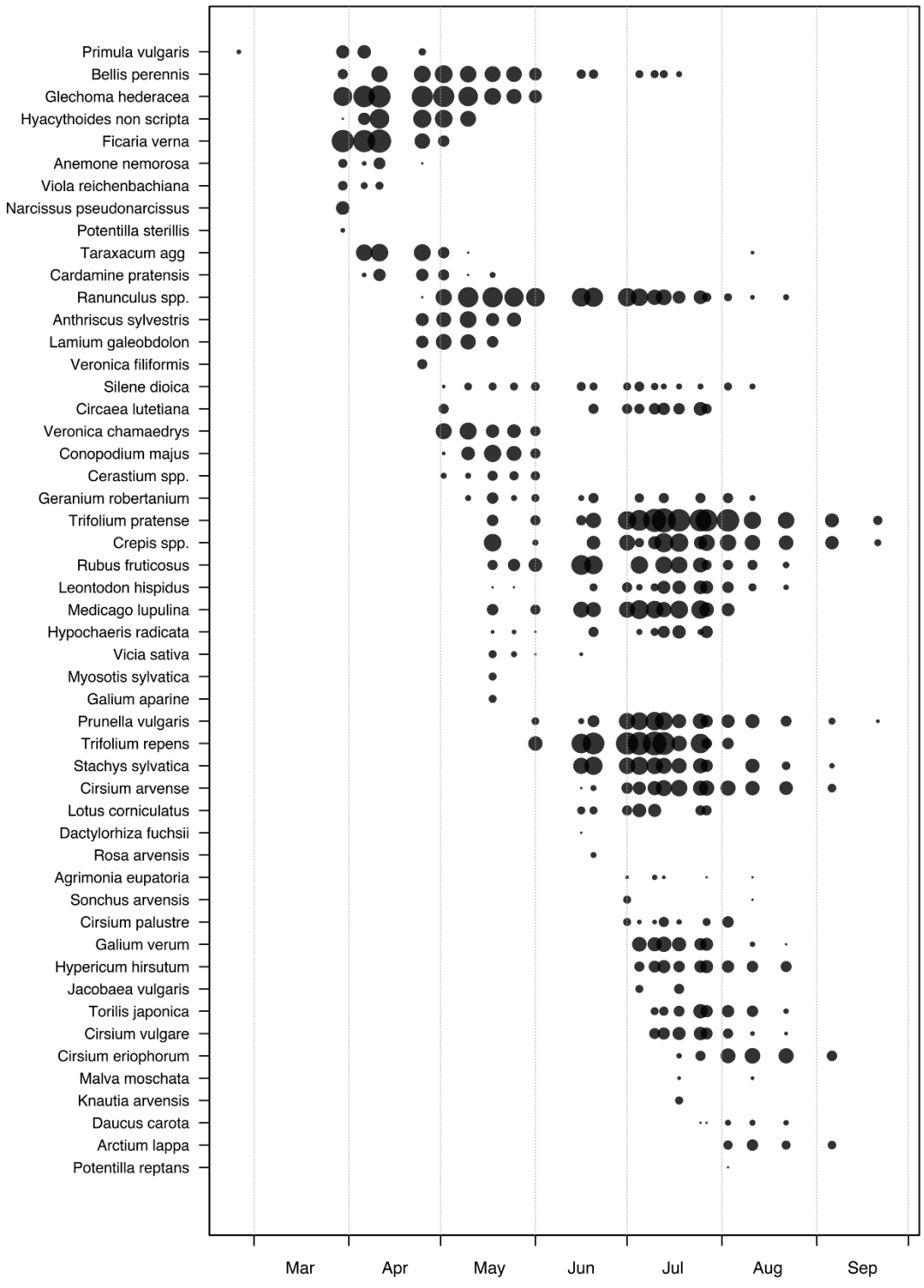


Figure 3.13 Flowering phenologies from T3 in 2017 showing progression of flowering species.

In Figure 3.14 we compare the total floral resource and the number of species present over time for the three habitats and in 2016 and 2017. There are a few points to note. The total floral resource, measured in floral units (FU), is variable over time across the three sites. In 2016, the total floral resource on T1 peaked at over 10,000 FU for a short period in June. In comparison the total floral resource peak was much lower on T2 (less than 2,000 FU) and T3 (less than 6,000). In 2016, the golf course rough was cut on the 22nd of July which impacted the total floral resource on T1 for the rest of the season: the total floral resource was declining on T1 prior to being cut and remained low after, whereas on in 2017 there was a second peak in total floral resource. It is possible that the cutting supressed a second peak in flowering in 2016. The distribution of total floral resource is double peaked for T1 in 2017, T3 in 2016 and T3 in 2017, with a dip in FU in June or July. This may be a phenomenon called the 'June Gap'; a period of fewer flowering plant species as spring flowering plants finish and before summer flowering plants start to peak (Balfour *et al.*, 2018). On T2 in 2016, there was also a reduction in FU in June, and FU stayed low for the rest of the season. This is most likely due to the tree cover which mean that by mid-June all of T2 was in shade. On the 26th of July in 2017, we cut all of the flowering heads of *Cirsium eriophorum* (woolly thistle) in the north area of Bushey Norwood, as part of the experiment detailed in Chapter 4. Only small fraction of the *C. eriophorum* flowers cut would have been included in the phenology transect survey and this does not appear to have significantly impacted the phenology data.

In contrast with the variability in FU, the number of plant species flowering at any given time was similar across habitats, peaking at 10-20 species in 2016 and 15-25 species in 2017. On T3 in 2016, there was a sudden dip in the number of flowering plant species, corresponding with the potential 'June Gap'.

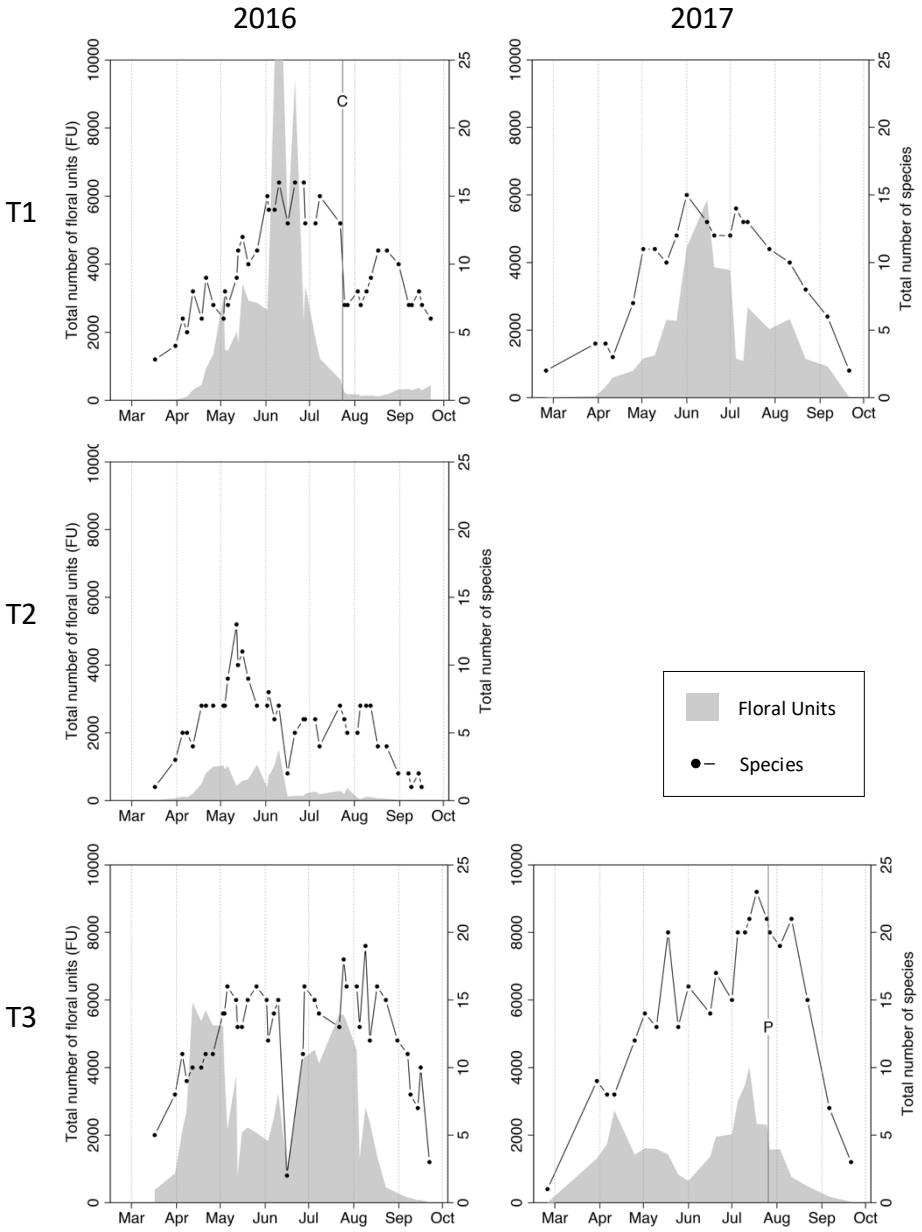


Figure 3.14 A comparison of the total number of species (black dotted line) and the total number of floral units (grey area) surveyed over time on the three sections of the transect (T1, T2 and T3 – rows) and over the two years (2016 and 2017 – columns). In T1-2016 the vertical line labelled C indicates that between the surveys on the 22nd and 25th of July the golf course rough was cut. In T3-2017, the vertical line labelled P indicates that on the 26th of July we cut all the flowering heads of one plant species (Cirsium eriophorum) as part of the experiment detailed in Chapter 4.

3.3.b Bumblebee phenology results

In 2016, we recorded 9 species of bumblebee and in 2017 we recorded 7 species, (counting the grouped *Bombus lucorum/terrestris* as a single species). These records are summarised in Table 3.4. We recorded two cuckoo bumblebees (*Bombus campestris* and *Bombus rupestris*), and one rare species only observed 3 times (*Bombus ruderarius*). For the majority of our analysis we will focus on the more abundant bumblebee species. We present bumblebee phenologies for the six most abundant bumblebee species in Figure 3.15. We do not separate records according to site because we assume bumblebees are able to move relatively freely between the sites.

Table 3.4 Summary of the total number of records for each bumblebee species in 2016 and 2017. Counts refers to the number of individual records. Two cuckoo bumblebee species were observed (*). Bombus terrestris queens (which can be visually separated from Bombus lucorum, unlike the workers) are listed separately here though combined as Bombus lucorum/terrestris for analysis (†). No Bombus lucorum queens were identified.

Bumblebee species	***Count 2016***	***Count 2017***
*Bombus. campestris **	*7*	*2*
Bombus hortorum	*43*	*9*
Bombus hypnorum	*18*	*5*
Bombus lapidarius	*174*	*64*
Bombus lucorum/terrestris	*100*	*47*
Bombus pascuorum	*133*	*59*
Bombus pratorum	*101*	*14*
Bombus ruderarius	*3*	*0*
*Bombus rupestris **	*1*	*0*
Bombus terrestris †	*13*	*0*

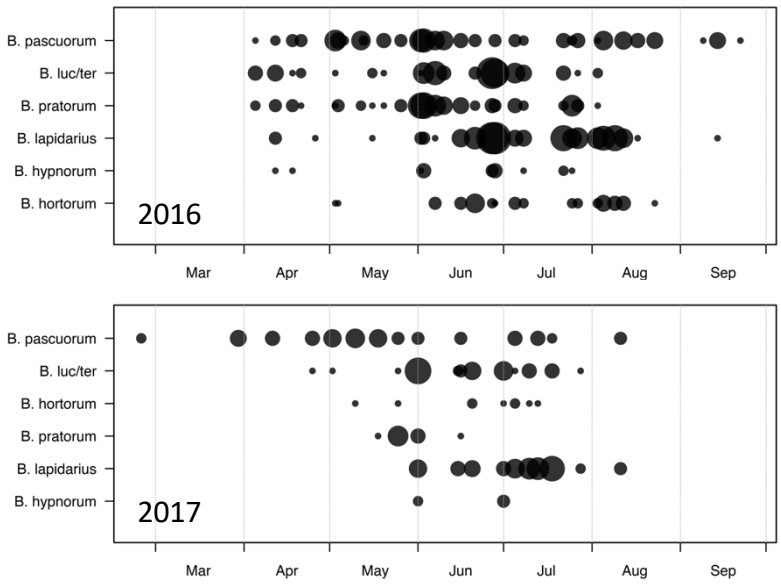


Figure 3.15 Phenologies of the abundant bumblebee species observed foraging along the transect in 2016 (top panel) and 2017 (bottom panel). Each point indicates when a bumblebee species (listed on the y-axis) was observed foraging. The radius of each point is scaled to the number of observations (log_2(observations + 1)) – note this is a different scale to that used for plant species plots.

3.4 Analysis methods

3.4.a Variation in flowering phenology and dominant species

We carried out a simple comparison of the flower phenologies from the different habitats to determine how similar the two consecutive years were. We expected that despite some fluctuations, plant species would peak at roughly the same time and at roughly the same number of floral units in 2016 and 2017. Using the records of plant species recorded in both years, we carried out a Spearman's rank correlation test to determine if the order in which plant species reached peak flowering was correlated in 2016 and 2017. We also compared the number of floral units recorded at the flowering peak for each species in 2016 and 2017. We compared peak abundance between the two years on a log_{10} scale and used a simple linear regression using the data from 2016 to model the data from 2017. This enabled us to determine the variation in the relative timing of peak flowering between the two years.

Often plant pollinator networks are dominated by plant species that are abundant in floral units and the number of interactions they receive, such as *Daucus carota* (wild carrot) in the Ashton Count network analysed in Chapter 2. We wanted to know if there were

dominant plant species on our transect that could potentially dominate a plant-pollinator network sampled at the site. We looked for dominant species in our data (FU greater than 1000 in any survey) and compared T1 and T3 in 2016 and 2017 to see if dominant species were consistent across the sites and the two years.

3.4.d Turnover of flowering plant species

In order to determine how representative a single survey was of the plant species flowering at the site compared to the whole season, we calculated the fraction of plant species present in each survey out of the total species present over the whole flowering season (for each section of the transect and in each year).

We also analysed species turnover between each survey day by calculating beta diversity between sequential pairs of survey days using the function 'betadiver' from the package 'vegan' (Oksanen *et al.*, 2017) in R (R Core Team, 2017). We used a common measure of beta-diversity, developed from Whittaker's original measure (Whittaker, 1960) and recommended by Koleff, Gaston and Lennon, (2003). This measure quantifies the difference in species present between 2 samples (in our case 2 survey days) using the equation:

$$\beta_w = \frac{a + b + c}{(2a + b + c)/2} - 1$$

where $a =$ the total number of species that appear in both samples, $b =$ the total number of species that appear in the second sample only and $c =$ the total number of species that appear in the first sample only. β_w lies between 1 and 0 where 1 is complete turnover (the species present in sample 1 and 2 are entirely different) and 0 is no turnover (sample 1 and 2 are identical). We calculated β_w between each sequential pairs of survey days (i.e. survey 1 vs survey 2, survey 2 vs survey 3 etc).

3.4.b Comparison of bumblebee and flowering phenologies

We were interested to know how bumblebee phenology compared to flowering phenology; were bumblebees foraging at the site for longer than the average flowering period of plant species? If the bumblebee phenologies are longer than the average flowering phenology, then bumblebee species must be adapting to changes in the floral resource available over time. We calculated the flowering period (the first to last observation) in days for each plant species and the foraging period (the first to last observation) for bumblebees,

using all records (in 2016 and 2017) from the transect combined. We tested to see if plant flowering periods were on average shorter than bumblebee foraging periods using a Mann-Whitney U-test.

We also wanted to know when bumblebee species were observed foraging on a plant species in comparison to first flowering and peak flowering of the plant species. We calculated the number of days between the first flowering record and the first bumblebee interaction recorded for all plant species on each section of the transect and in each year and the number of days between peak flowering and the first bumblebee interaction.

4.3.c Skewness of flowering phenologies

We wanted to examine the skewness of the flowering phenologies as the skewness of overlapping plant phenologies has the potential to impact pollinators that feed on those plant species (see Figure 3.16). We calculated the skewness of the flowering phenology of all plant species (present on at least three survey days) using the function 'skewness' from the package 'moments' (Komsta and Novomestky, 2015) in R (R Core Team, 2017). We used Spearman's rank to test for a correlation between skewness and first day of peak floral units to see if skewness was linked to when in the season a plant species flowered. We also used Spearman's rank to test for a correlation between skewness and maximum floral units to determine if skewness was linked to the flowering abundance of plant species.

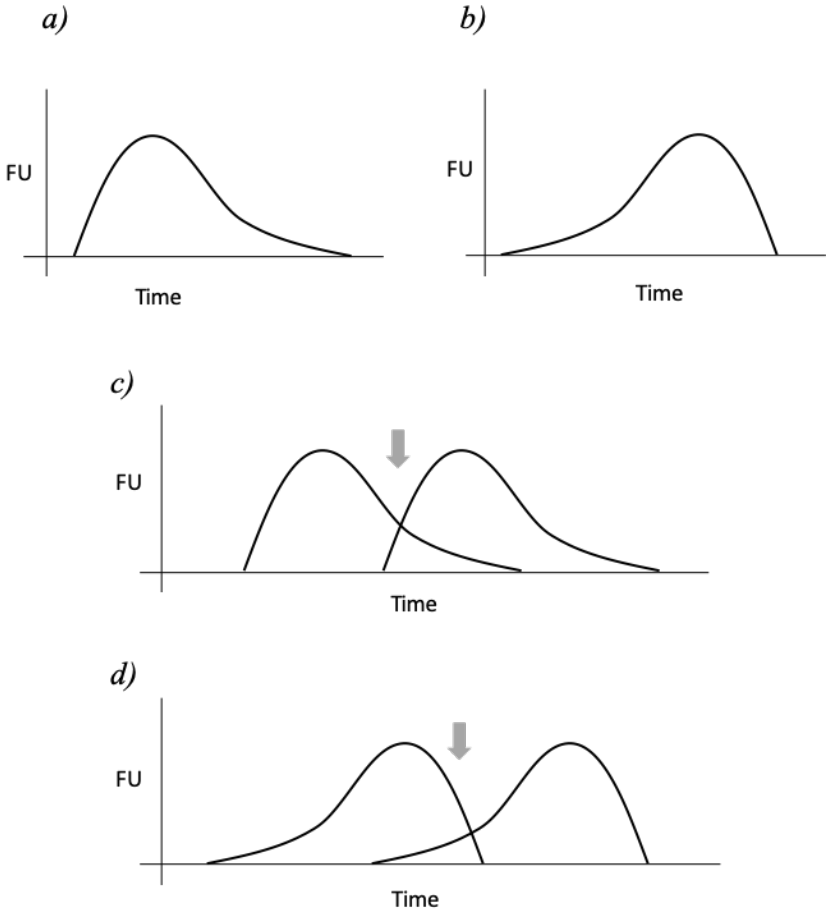


*Figure 3.16 Schematic demonstrating **a)** positive and **b)** negative skew of the flowering phenology of a plant species. **c)** If most plant species have positively skewed phenologies then in the period of overlap (indicated by the grey arrow), pollinators have to switch from a slowly declining resource to a suddenly abundant, new resource. **d)** Alternatively, if most plant species have negatively skewed phenologies then in the period of overlap (indicated by the grey arrow) pollinators have to switch from a rapidly declining resource to a gradually increasing new resource.*

3.5 Results

3.5.a Variation in flowering phenology and dominant species

The floral resource was not consistent over 2 years; 31-34% of plant species were only present in one year. On T1, 27 plant species were recorded in both 2016 and 2017 and 14 were recorded in only one year. On T3, 42 plant species were recorded in both years and 19 were recorded in only one year. However, there was some consistency in flowering progression for species that were present in both years. We found that the first day of peak

floral units of each plant species was significantly positively correlated between 2016 and 2017; on T1 $\rho = 0.830, p = 8.58 \times 10^{-8}$ and on T3 $\rho = 0.895, p = 1.39 \times 10^{-15}$ (See Figure 3.17).

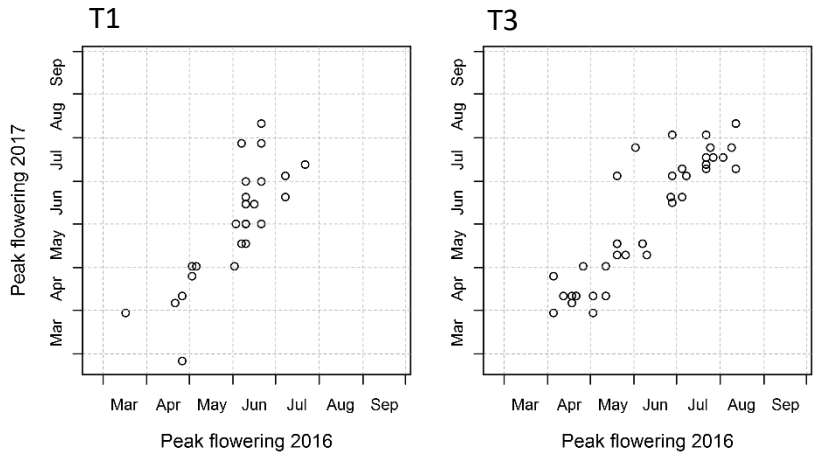


Figure 3.17 Comparison of the first day flowering (measured in floral units) for each plant species in 2016 and 2017 on T1 (left hand plot) and T3 (right hand plot).

Plant species peaked with more floral units in 2016 compared to 2017 and a significant linear relationship was found between peak FU in the two years (Figure 3.18). Peak floral units in 2017 (measured on a log10 scale) were modelled according to the equation:

For site T1: $log_{10}(Peak\ FU\ 2017) = 0.87(log_{10}(Peak\ FU\ 2016))$,

F(1,26) = 553.6 and $p < 2.2 \times 10^{-16}$,

For site T3: $log_{10}(Peak\ FU\ 2017) = 0.88(log_{10}(Peak\ FU\ 2016))$,

F(1,41) = 323.6 and $p < 2.2 \times 10^{-16}$

On average, therefore, the log-transformed peak FU in 2017 were, on average, 0.87-0.88 times the log-transformed peak FU in 2016, and this effect was consistent between sites. This means that on T1, for every 100 FU of a plant species recorded in 2016 there were on average 55 FU in 2017; on T3 for every 100 FU recorded in 2016 there were 58 FU in 2017. In both cases, the slope of the regression, was significantly different to 1. The 95% confidence intervals of the slope of the regression were: 0.797, 0.945 for site T1 and 0.786, 0.983 for site T3. R^2 was equal to 0.95 and 0.88 for T1 and T3 respectively suggesting that our model is a good fit.

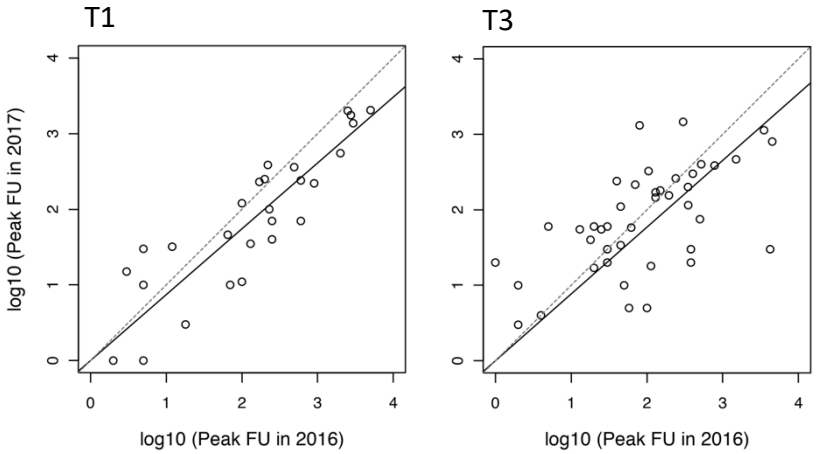


Figure 3.18 A comparison of the peak number of floral units recorded for each plant species in 2016 and 2017 on T1 (left) and T3 (right). Peak FU in compared between the two years on a log10 scale with the regression $log_{10}(Peak\ FU\ 2017) = \beta(log_{10}(Peak\ FU\ 2016))$ *plotted with* $\beta = 0.87$*(standard error = 0.037) for T1 and* $\beta = 0.87$ *(standard error = 0.049) for T3. The line x=y (grey dashed line) is plotted for comparison.*

We found that on T1 and T3 there were clear dominant species and that these species tended to succeed one another as shown in Figure 3.19. Dominant species were consistent across years on both sections of the transect; the same species were dominant in 2016 and 2017. On both sections of the transect, *Ranunculus spp.* and *Trifolium pratense* were dominant. T1 had three other dominant species: *Leontodon hispidus, Rhinanthus minor* and *Taraxacum agg.* T3 had four other dominant species: *Cirsium palustre, Ficaria verna, Glechoma hederacea* and *Trifolium repens*. On both T1 and T3 (in both years), dominant species were fairly spread out and peaked roughly in a succession. All of these dominant species were observed being visited by bumblebees (see Figure 3.23).

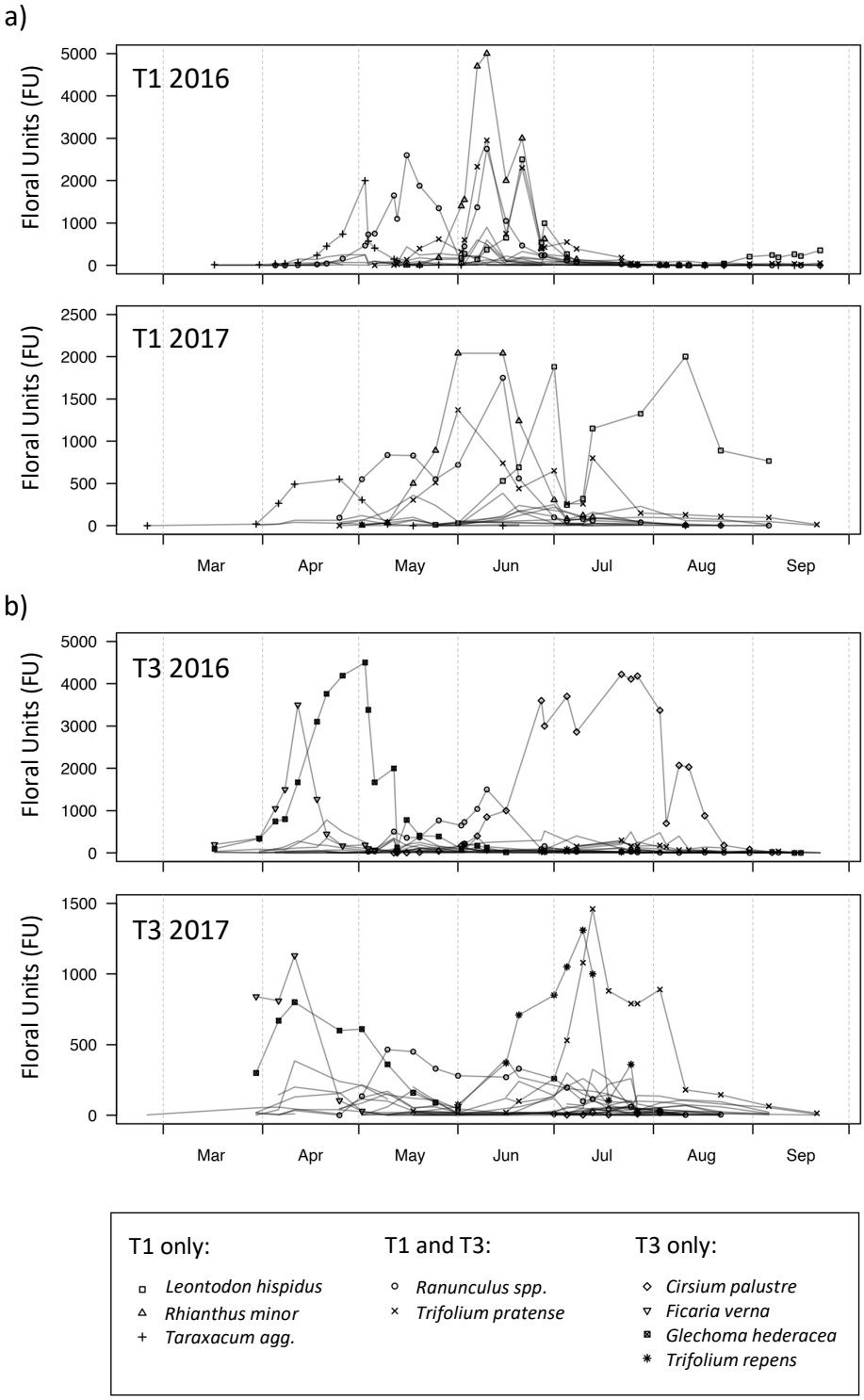


*Figure 3.19 Flowering phenologies showing dominant plant species (see key) that reached >1000 FU in at least one survey compared across **a)** T1 and **b)** T3, both in 2016 and 2017. Dominant species were consistent across years on both sections of the transect.*

3.5.b Turnover of flowering plant species

We found that overall, the fraction of flowering plant species recorded in a single survey day compared to all plant species over the whole flowering season (on each section of the transect and in each year) was low as shown in Figure 3.20. Only on one occasion did the fraction of plants recorded in a single survey reach over 50% of the total (on T2 in May 2016). The majority of surveys recorded between 20-40% of plant species and some recorded fewer than 10%, particularly early in the season (February-March) and late in the season (September) though also in June corresponding with our possible 'June Gap'. The best time to survey in terms of the most species represented depended on the habitat. The fraction of species represented was highest on T1 in June (2016 and 2017), T2 in May (2016) and T3 in August (2016) and July (2017). However, if we take species turnover in account also, the story is more complex. Figure 3.21 shows species turnover, measured as beta diversity, over time. Overall, mean species turnover between consecutive surveys on the transect as a whole (T1, T2 and T3 combined) was 0.198 in 2016 (standard deviation $= \pm 0.144$) and 0.247 in 2017 (standard deviation $= \pm 0.148$). In 2017, turnover was fairly constant between 0.1 and 0.4. On the other hand, in 2016 both T2 and T3 had peaks turnover in June at 0.55 and 0.85 respectively. That mean between two consecutive surveys there was a 55% and 85% turnover in plant species flowering on T2 and T3. This suggests that collecting data for plant-pollinator networks either side of this peak would result in networks with very different plant species, and therefore presumably different plant-pollinator interactions.

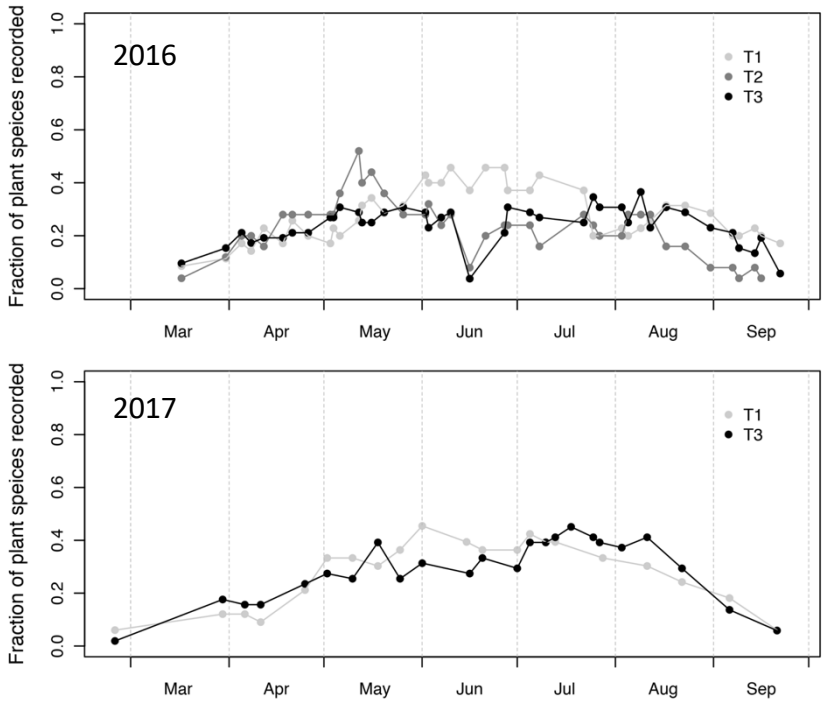


Figure 3.20 The fraction of species recorded in survey out of the total number of species recorded over the season in 2016 (top panel) and 2017 (bottom panel).

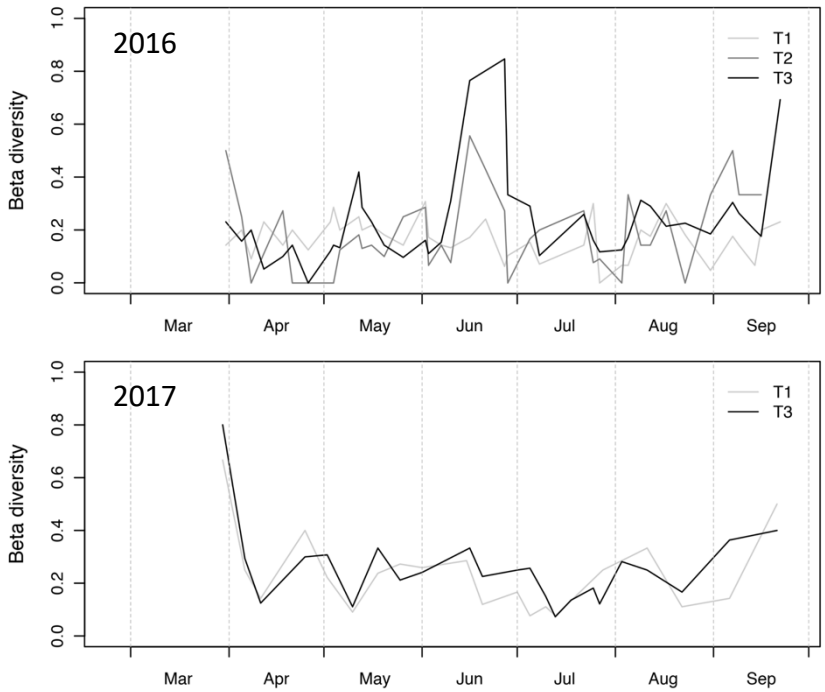


Figure 3.21 Beta diversity of the plant species flowering over time on each section of the transect in 2016 (top panel) and 2017 (bottom panel).

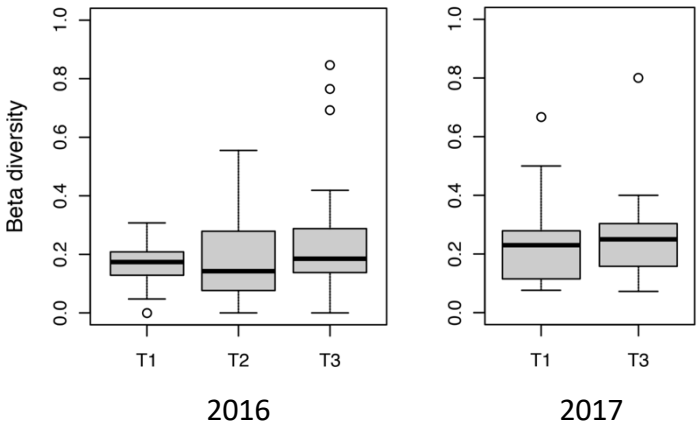


Figure 3.22 Boxplot summary of the beta diversity for each section of the transect in 2016 (left hand panel) and 2017 (right hand panel).

3.5.b Comparison of bumblebee and flowering phenologies

We found that bumblebee phenology was on average significantly longer than plant phenology: $U = 498.5, p = 0.0004$. Bumblebee species at the site outlived the flowering period of most plant species, indicating that they must forage on different plant species over a season. This can be seen when comparing the bumblebee phenologies in Figure 3.15 and the flowering phenologies in Figure 3.9 to Figure 3.13.

We found that on average bumblebee species were observed interacting with plant species 18 days after first flowering was recorded (standard deviation $= \pm 14$), and on average 18 days before peak flowering (standard deviation $= \pm 17$). These results are broken down by section of the transect and year in Table 3.5 and Table 3.6. Even accounting for the fact that the mean number of days between surveys was 4.7 in 2016 and 7.7 in 2017, these results suggests that there was a lag between bumblebees interacting with plant species after they had first flowered, but that on average bumblebees started interacting with plant species before they reach peak flowering (Figure 3.23).

Table 3.5 Summary of the lag times between first flowering observation and first bumblebee interaction observed (in days). For both years and the whole transect combined: mean = 18 with standard deviation = ±14.

		Period between first flowering and bumblebee interactions (days)			
Year and site		**Mean**	**Median**	**Max.**	**Min.**
2016	T1	19	19	46	0
	T2	18	19	42	0
	T3	10	14	16	1
2017	T1	16	19	37	0
	T3	21	15	71	0

Table 3.6 Summary of the lag times between first flowering observation and first bumblebee interaction observed (in days). Negative values indicate that the first bumblebee interaction was observed before peak flowering. For both years and the whole transect combined: mean = -18 with standard deviation = ± 17.

		Period between peak flowering and bumblebee interactions (days)			
Year and site		**Mean**	**Median**	**Max.**	**Min.**
2016	T1	-4	0	-37	25
	T2	-12	-12	-23	0
	T3	-12	-8	-57	5
2017	T1	-15	-13	-71	0
	T3	-11	-13	-46	15

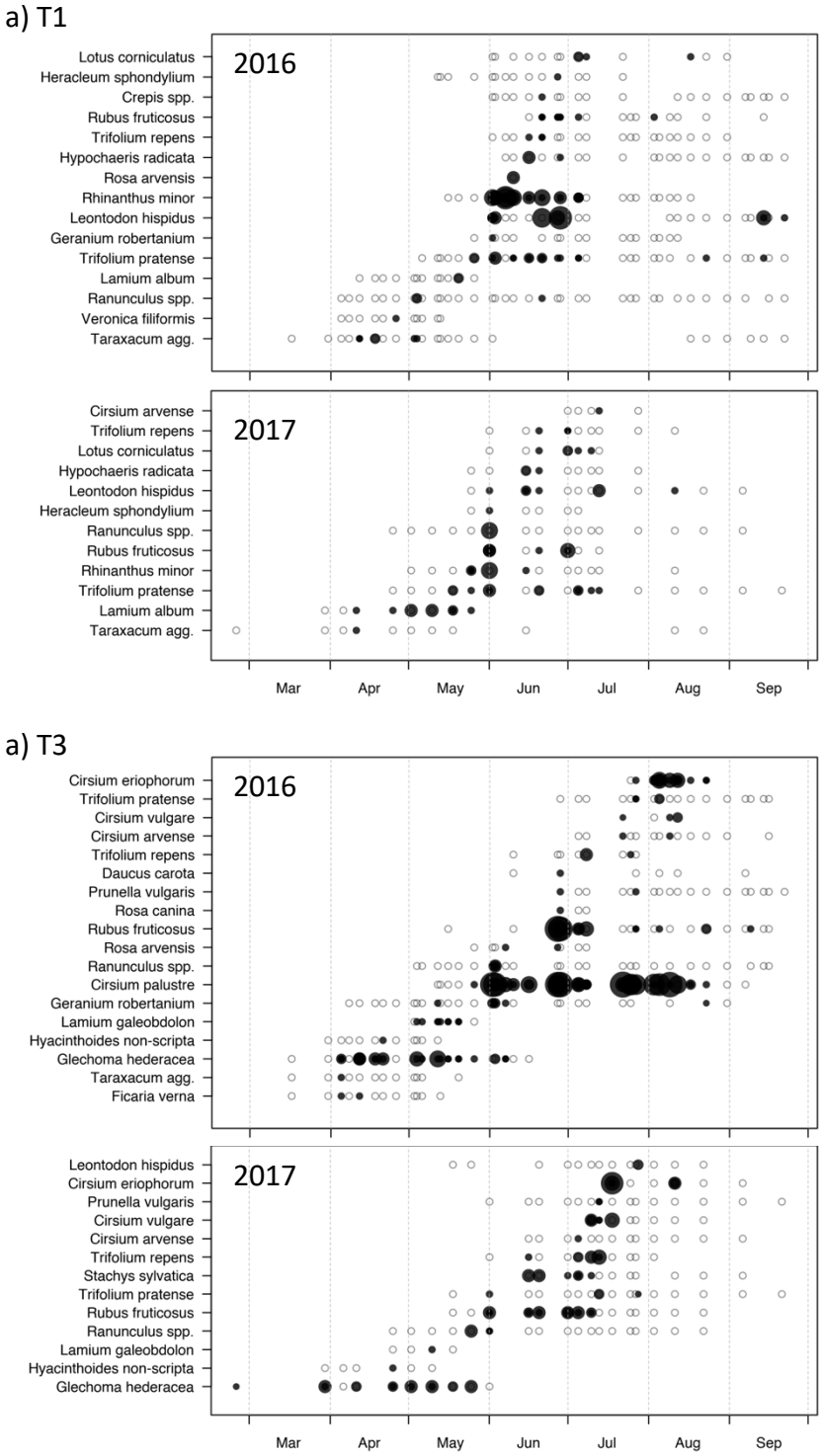


*Figure 3.23 Interactions between bumblebee species and plant species on **a)** the T1 section of the transect and **b)** the T3 section of the transect, in 2016 and 2017. White points indicate when a plant species was observed flowering. Black points indicate when an interaction(s) was observed between a bumblebee(s) and the corresponding plant species. The radius of each black point is log_2 scaled to the number of interactions observed (log_2(no. of interactions+1)).*

4.3.c Skewness of flowering phenologies

The majority of plant species had positively skewed phenologies (see Figure 3.24). Of the 160 flowering phenologies analysed, 128 (80%) were positively skewed, 17 (11%) were negatively skewed and 15 (9%) were not skewed. There was no significant rank correlation between skewness and peak flowering day: $\rho = -0.039, p = 0.617$. However, there was a significant, weakly positive rank correlation between skewness and peak floral units: $\rho = 0.499, p = 1.78 \times 10^{-11}$. This suggests that the plant species with a greater peak in floral units are more likely to be more positively skewed, and only plant species with a relatively small peak in FU had a negative skew.

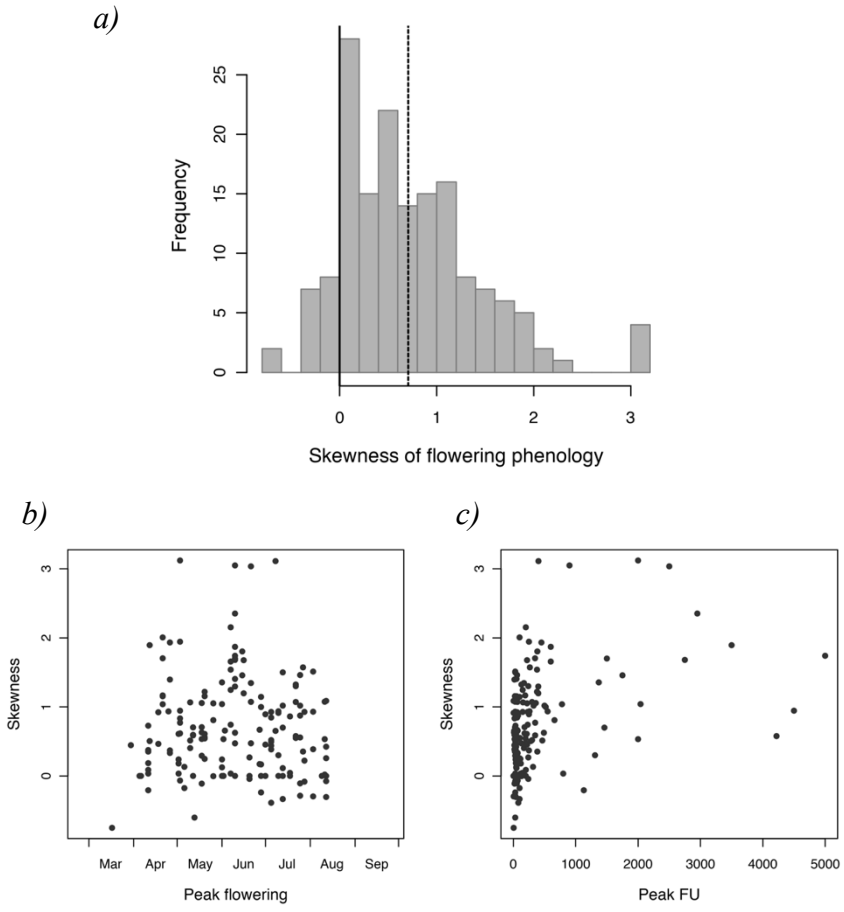


Figure 3.24 ***a)*** *Histogram showing the skewness of plant flowering phenologies for all 160 phenologies (that had at least 3 records). The dashed line indicates the mean skewness.* ***b)*** *There was no significant rank correlation between skewness and peak flowering day.* ***c)*** *There was a significant, positive rank correlation between skewness and peak floral units, though variation was high (FU):* $\rho = 0.499, p = 1.78 \times 10^{-11}$.

3.6 Discussion

Accounting for the dynamic nature of plant-pollinator communities in plant-pollinator network data is not an easy task. It is however, important to consider the dynamics and variation in plant-pollinator communities over time, especially when sampling for network data and using network data in further analysis of plant-pollinator communities. Recent advances in this field are promising (Kaiser-Bunbury *et al.*, 2014; Poisot, Stouffer and Gravel, 2015; Biella *et al.*, 2017; CaraDonna *et al.*, 2017). The results presented in this chapter highlight the continuous changes in species turnover at our field site throughout the pollination season.

We analysed the dynamics of the plant-pollinator community at our field site using various measures. We focused on flowering phenology and showed how the floral resource available to insect pollinators changed over time. Flowering phenology varied across the three different habitats observed and ~32% of plant species were only present in one of two consecutive years. This is likely due to the spatially narrow sampling, though some plant species have dormant years when they do not flower as part of their natural lifecycle. Plant species could also be missed if they had a very short flowering period which was missed between survey days. Despite this variation, the order of flowering progression was fairly consistent for plant species present in both years, in agreement with CaraDonna *et al.* (2017). We found it was possible to predict the abundance of flowering species (measured in floral units) in 2017 from the 2016 data, though overall floral units were significantly lower in 2017.

Plant-pollinator networks are often dominated by highly abundant and highly visited plant species (Vázquez, Chacoff and Cagnolo, 2009; James, Pitchford and Plank, 2012) as we found in the networks analysed in Chapter 2 (see for example Memmott, 1999). We found that the sections of our transect were dominated by a small number (4-5) of highly abundant species but that at any given time there tended to be only one dominant species present. This indicates the importance of sampling period for plant-pollinator network data as highly abundant species might be mistakenly considered 'important' in the community without consideration of the turnover of abundant species.

In any given survey, the fraction of plant species flowering compared to the whole season was low (20-40%). Early and late in the season ~10% of species were represented. The 'best' time to survey in terms of the greatest number of plant species represented differed between the three habitats. The 'best' survey time was also confounded by plant species turnover. Overall, the turnover of plant species between consecutive surveys was

fairly consistent (0.198 in 2016 and 0.247 in 2017), however in two of the three habitats represented by our transect there was high turnover in June, suggesting a turnover from spring to summer flowering plant species.

Bumblebee foraging observations are less reliable than floral unit observations on a fixed transect. If a plant species is flowering it will be recorded, and so the only unknown flowering period is between the survey day that it was first recorded and the one prior to that when it was not yet flowering. However, bumblebees are not limited to the survey area and are likely to be constantly moving around. It is possible that bumblebee individuals were present at the site, but not missed in the survey, or that were foraging at a nearby site and so not included in the survey. In addition, note that the phenology of a species, as estimated here, may include observations of individuals from multiple colonies and it is known that colonies of the same species can have varied phenologies (Goulson, 2010). Despite these limitations our results show that bumblebee phenologies were significantly longer than flowering phenologies and therefore bumblebees must adapt their foraging patterns based on the changing floral environment. We also found that the majority of plant species were first visited by bumblebees before the plant species reached peak flowering. Overall, our results suggest that bumblebees are flexible foragers and are able to adapt to shifting flowering phenologies. This is a key point and one that we will turn our attention to in Chapter 5.

Chapter 4

A pulse perturbation to a plant-pollinator network

Abstract

In order to survive, pollinators must be able to adapt to changes in available floral resources. We created a field-based temporary knock-out extinction scenario (a pulse perturbation), to collect empirical evidence for i) the effect of species removal on the plant-pollinator community as a whole and ii) the effect of species removal on the foraging behaviour of specific pollinator species. We removed the flowers of an abundant plant species, *Cirsium eriophorum,* from a plant-pollinator community at a species-rich site. We sampled plant-pollinator interactions from the site 1 week before and immediately before the removal of flowers. We sampled immediately after the removal of flowers and once *C. eriophorum* had re-flowered (1 week after removal). We assessed how the structure of the network changed in response to the pulse perturbation in terms of standard network metrics. In order to determine the direct effects of the loss of *C. eriophorum,* we compared the foraging patterns of three bumblebee species that were strongly associated with *C. eriophorum* before and after the perturbation. We quantified changes in the foraging patterns of all species to determine if there were indirect effects of the perturbation that spread through the network. We found that:

a. The removal of *C. eriophorum* did significantly change the network structure but the network 'healed' after a period of 7 days.
b. The three most abundant bumblebee species at the site had distinct and different responses to the loss of *C. eriophorum*. After re-flowering, the bumblebees more-or-less returned to their pre-perturbation foraging patterns.
c. There is evidence for indirect effects that spread through the network as a result of the perturbation. The magnitude of changes to plant and pollinator interactions in the network can be predicted based on their distance in the network from *C. eriophorum.* The actual value of change can be predicted for plant species degree and interactions using weighted distance but not for pollinator species as variation in the type of response was high.

4.1 Introduction

The floral resources available to insect pollinators change over time. As we saw in Chapter 3, the flowering phenologies of different plant species overlap; at any given point in time, there is a different foraging environment (Tooke and Battey, 2010). Many pollinating insects have individual and colony lifespans long enough that they will live through changes to their foraging environment (Solga, Harmon and Ganguli, 2014). In order to survive, individuals and species must be able to respond to these changes. An inability to respond to anthropogenic changes in flowering phenology and loss of plant species is generally considered to be one of the factors behind declines in pollinator species (see Memmott *et al.*, 2007; Hegland *et al.*, 2009 for an overview). Specifically, studies have shown the climate change can cause the temporal mismatch of species as the lifecycle timing of different species shift at different rates (Burkle, Marlin and Knight, 2013; Schenk, Krauss and Holzschuh, 2018). There is also evidence for the spatial mismatch of species as a result of climate change (Schweiger *et al.*, 2008). These studies highlight the importance of the co-occurrence of plant and pollinator species that benefit each other, though do not address what level of variation in floral resources are can be tolerated by pollinator species. Understanding how pollinators respond to changes in floral resources can inform conservation practices and help to mitigate the potential effects of the loss of plant species.

Existing approaches for studying plant-pollinator networks can be roughly divided into two categories; observational, descriptive studies and theoretical modelling techniques. There are many examples of descriptive studies that have used field observations to build datasets of plant pollinator networks (see for example; Memmott, 1999; Dunne, Williams and Martinez, 2002). Descriptive studies have been used to inform theoretical approaches that model changes to plant pollinator communities. In Chapter 2, we examined knockout extinction models which calculate the robustness of plant-pollinator networks to hypothetical extinction scenarios based on knowledge of the biology of plant-pollinator communities and using observational data. Despite recent increases in descriptive and theoretical approaches, there is a lack of empirical evidence regarding the temporal dynamics of plant-pollinator networks and the effect of species extinctions on the community as a whole over both short (daily and within a season) and long (yearly and beyond) timescales. Delmas *et al.*, (2018) suggest comparison of networks sampled from the same community over time as an area requiring further exploration and point out the lack of this kind of data. In addition, it is clear from the dynamic plant and pollinator phenologies discussed in Chapter 3 that plant-pollinator networks are not static and modelling from single snapshot network data can therefore be limited. More empirical studies are needed in order to determine experimentally

the effects of species loss on dynamic communities. In general, there are few studies that have experimentally manipulated ecological networks of any kind.

One of the first studies to experimentally manipulate an ecological network (though they use the term food web) was that by Hanson and Butler (1994). In 1987, they experimentally removed fish from a shallow lake that was an important feeding area for migrating diving birds. They detected responses (monitored from 1987 to 1990) in water clarity, plant growth, macroinvertebrate density and use by diving birds, demonstrating the far-reaching effects of species removal over 3 years. To the best of our knowledge, there are only 5 published studies that experimentally remove or introduce a species in the field and examine the effect on the mutualistic networks that describe the community in question. The first of these looked at the impact of an alien invader (*Impatiens glandulifera*) on a native plant-pollinator network in terms of species richness, competition and visitation (Lopezaraiza-Mikel *et al.*, 2007) by comparing *I. glandulifera* invaded plots and plots where *I. glandulifera* was experimentally removed. They found that *I. glandulifera* invaded plots had significantly higher pollinator species richness, pollinator abundance and flower visitation, but were dominated by alien pollen and therefore potentially had lower pollination success for native species. The second study focused on the effect of removing the dominant seed-dispersing ant species from a seed dispersal networks in three habitats (Timóteo *et al.*, 2016). They found that the network rewired, with the remaining ants widening the breadth of their diet and maintaining seed dispersal. They found that a simulation model considerably overestimated the effect of species loss compared to the empirical data. In the same year, Goldstein and Zych published their study in which they removed inflorescences of the most connected plant species in a plant-pollinator community and compared the results to simulated and control scenarios. Unlike other studies, they found the network did not significantly change in terms of common network metrics and no secondary (knock-on) extinctions were observed (Goldstein and Zych, 2016). On the other hand Brosi, Niezgoda and Briggs (2017) experimentally removed the most abundant bumblebee species from multiple experimental plots and measured the effect in terms of numerous network metrics. They found that some network metrics (niche overlap, connectance and niche partitioning) reflected changes in the network structure as a result of the experimental manipulation whilst others (nestedness and specialisation) did not change. Most recently, Biella *et al.* published their experimental removal of generalist plant species in a plant-pollinator community (paper accepted, pre-print available on BioRxiv: Biella *et al.*, 2018). They measured the impact of the removal on flower visitation, pollination effectiveness and insect foraging finding that overall, visitation decreased and that the level of pollinator response to the manipulation was

limited by floral traits. Overall, these studies suggest some network level effects as a result of experimental manipulation. Brosi, Niezgoda and Briggs (2017) call for more manipulative experiments that investigate further indirect effects and integrate theoretical predictions.

To address this, we designed a 'pulse perturbation' experiment to determine the effect of the temporary removal of a key plant species on a plant-pollinator network. We chose to remove a key plant species as we were interested in the response of pollinators to the loss of a plant, given the seasonal changes in plant phenology examined in Chapter 3, and it was practically simpler than removing a pollinator species in a non-closed experimental site. Having collected plant and bumblebee phenology data throughout the spring and summer of 2016 at our field site, we had good knowledge of the species present and a rough idea of the changes in their abundance over time. We used this knowledge to select a key plant species, *C. eriophorum* as our target species. We were able to anticipate its flowering and survey the plant-pollinator community as it began to flower and once it had become a major resource for some pollinator species. Once it had reached near peak abundance, we removed all flowering heads and immediately surveyed to determine the effect of removal on the network. We then allowed a short period of time for *C. eriophorum* to re-flower to pre-manipulation abundance and then surveyed after re-flowering. We used the data from each of the four surveys to build four snapshot plant-pollinator networks that describe the plant-pollinator interactions at the key time periods around the perturbation. The primary aims of this study were to determine:

i) if and how the network structure changed as a result of the manipulation,
ii) what were the direct effects of the removal of *C. eriophorum* on the species that were strongly associated with it (3 bumblebee species),
iii) if there were indirect effects of the loss of *C eriophorum* that cascaded through the network to other plant and pollinator species.

We hypothesised that the network structure would change as a result of the perturbation, in agreement with previous work discussed above. We also hypothesised that the three bumblebee species would have to react to the loss of a major foraging resource by either switching to feed on other plant species, or leaving the site to forage elsewhere. To the best of our knowledge this is the first experimental study to also examine the effect of re-flowering (after removal of a plant species) on bumblebee species in the context of a real plant-pollinator community. Finally, we hypothesised that the removal of *C. eriophorum* would have indirect effects on other plant and pollinator species in the network via a cascade of effects as predicted in theoretical knock-out extinction models in Chapter 2 (Pocock, Evans and Memmott, 2012; Vieira and Almeida-Neto, 2015; Kaiser-Bunbury *et al.*, 2017;

Traveset, Tur and Eguíluz, 2017). We will address each of these hypotheses, in order, in this chapter.

4.2 Experimental methods

4.2.a The survey site: Bushey Norwood

The experiment took place at Bushey Norwood, Claverton Down, Somerset, UK (51.3128° N, -2.3206° W), the same location as the T3 transect in Chapter 3. Bushey Norwood is a 68-hectare, parkland meadow, most of which is flat, calcareous grassland with mature oak and ash trees. We defined a study area, approximately 300m by 20m (~0.6 hectares), within Bushey Norwood along the northern boundary (See Figure 4.1 and Figure 4.2). Note that the transect in Chapter 3 lies within this study area but that in this chapter we are sampling from the whole area, not just along the transect. From the surveys in 2016 (Chapter 3) we knew that this area was rich in plant species, offered a variety of food sources for pollinators, and that there were many pollinator species present.

Figure 4.1 A map showing the general location of the survey area on Claverton Down, next to the University of Bath (© OpenStreetMap contributors, openstreetmap.org accessed 09.03.18). The survey area is within the area enclosed by the grey dashed line.

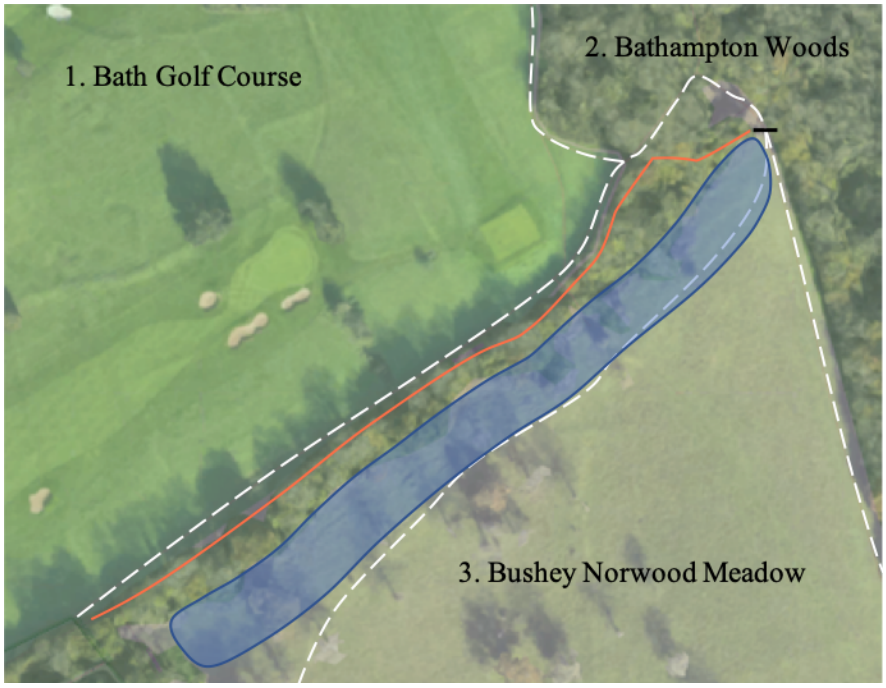


Figure 4.2 An aerial view of the survey area (blue shaded area). The white dashed line indicates the public footpath. The solid red line indicates the location of the wall boundary. The three surrounding habitats (golf course, woodland and meadow) are labelled. (Image composite from © OpenStreetMap contributors, openstreetmap.org accessed 09.04.18 and Imagery ©2018 Google, Map data ©2018 Google, accessed 09.04.18)

4.2.b The pulse perturbation and survey methods

Cirsium eriophorum (woolly thistle, See Figure 4.3) was chosen as the target plant species for the pulse perturbation for several reasons. Firstly, we knew from the phenology survey in 2016 (Chapter 3) that it was a key foraging resource and was visited my multiple pollinators (mainly *Bombus* species), though this is not seen in the data presented in Chapter 3 as *C. eriophorum* was mostly in large clusters of outside of the transect survey area. Secondly, each stem typically has multiple heads which flower at staggered intervals, allowing us to remove flowering heads without eliminating the ability of the plant to re-flower. We also knew from the phenology survey in 2016 the approximate flowering time and it was clear from visits to the site in early 2017 that the number of *C. eriophorum* plants was going to be high.

Figure 4.3 Cirsium eriophorum, commonly known as woolly thistle, in the early stages of flowering. A Bombus lapidarius worker can be seen feeding on thistle head.

Overall, we carried out 4 surveys; two before the removal of *C. eriophorum*, one immediately after, and a final survey after re-flowering of *C. eriophorum*. Each survey took place over 2 or 3 days. The first survey (called S1), took place on the 19th and 20th of July, when *C. eriophorum* flowers had begun to open and were present in small numbers; 43 were counted on the 20th. The second survey (called S2) was carried out on the 24th and 25th of July when open *C. eriophorum* flower numbers were estimated to be about half their peak based on the number of plants and buds present (282 open flowers counted on the 24th). On the 26th of July, the perturbation took place. All the flowering heads of the *C. eriophorum* plants in the survey area (317 in total) were cut off just below the flower head as demonstrated in Figure 4.4. This ensured minimal damage to the plant and any buds remaining on the plant. Although the plants remained, by cutting the flowers off we had effectively removed *C. eriophorum* so it could no longer be a foraging resource for pollinators at the site. To the best of our knowledge, there were no patches of *C. eriophorum* growing elsewhere in Bushey Norwood or within the surrounding habitats (within approximately 500m radius from the survey site). Having removed *C. eriophorum*, the third survey (called S3) took place on the 27th, 28th and 29th of July; at the beginning of each day, any newly-flowered *C. eriophorum* were cut. After S3, we checked the site each day to see how many had re-flowered. The final survey (called S4) was on the 3rd and 4th of August, when the number of *C. eriophorum* flowers had increased to 494. Figure 4.5 shows the actual survey periods and *C. eriophorum* counts as well as the schematic of our plan for the experiment.

Figure 4.4 Cutting a flowering C. eriophorum flower head off just below the flower head.

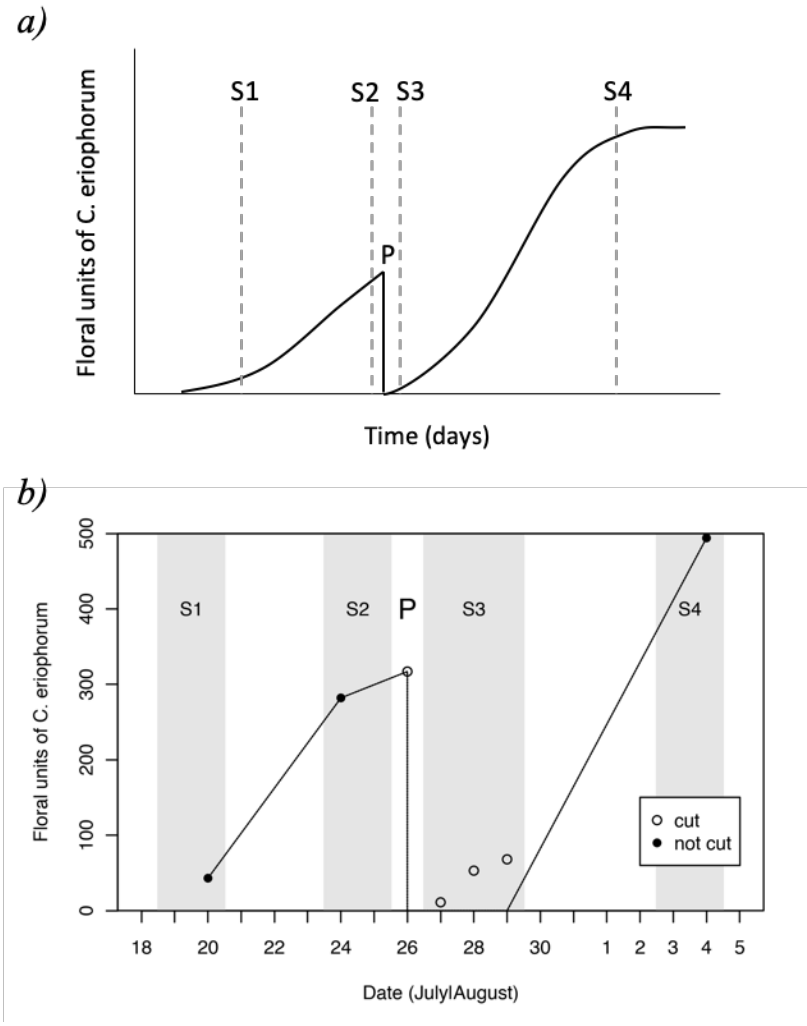


*Figure 4.5 A comparison of **a)** the expected population of C. eriophorum and the planned survey periods with **b)** the observed population of C. eriophorum and the actual survey periods. The population changed as expected. 'P' indicates the timing of the perturbation; when C. eriophorum flowers were removed. The open circles in the S3 survey period (b) indicate the number of C. eriophorum flowers that were cut and removed from the site before the survey began that day.*

For each interaction network survey, a single observer (Miranda Bane) recorded the plant-pollinator interactions observed whilst walking through the survey area. The observer walked at a steady pace, back and forth across the width of the area, with the aim of evenly sampling the whole area. An interaction was recorded only when a flower-visiting insect was observed making contact with the pollen and/or reproductive organs of a flowering plant. All pollinators surveyed (except for bumblebees and butterflies) were netted, killed using ethyl acetate, and preserved by freezing for identification at a later date. Individual bumblebees were netted and transferred to a 'bumblebee holding device' (See Figure 4.6) to be identified and marked on a wing using permanent pen before being released. Markings were not individual specific but a different colour was used to distinguish between the first two and subsequent surveys. Individual butterflies were also netted and marked on a wing with a permanent marker and then released.

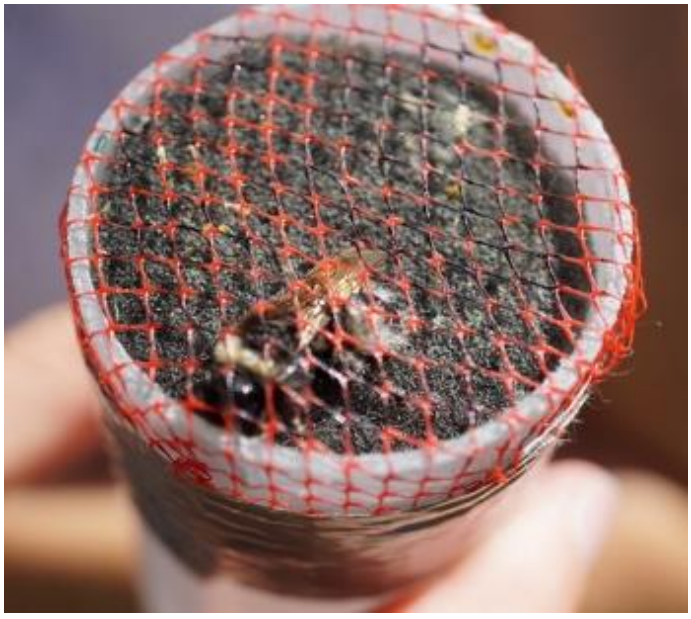


Figure 4.6 A bumblebee in the 'bumblebee holding device' ready to be marked with a coloured dot on its wing before being released.

4.2.c Plant and pollinator identification

Our aim in identification of plants and pollinators was to achieve high resolution (species level identification) where this was possible and efficient. Flowering plants were identified to species on site. Some species with very similar flower morphology and colour were grouped for efficiency when recording plants during the interaction surveys.

Specifically, *Ranunculus* species (*R. acris, R. bulbosus* and *R. repens*) were lumped, as were *Crepis* species (*C. capillaris* and *C. biennis*). *Taraxacum officinale* represents grouped micro-species. We decided to group these species because it would be time consuming to identify them to species level during the surveys and we wanted the time spent recording each interaction to be roughly equal. Of the 1468 individual insects recorded, we identified 1288 to species level (=88%). Pollinator specimens were identified by Miranda Bane and consultant entomologist David Gibbs (https://davidjgibbs.webs.com). Where species could not be identified because it was not possible in the field (in the case of bumblebees and butterflies), the specimens were damaged or it was not possible without DNA analysis, we lumped species. We made decisions about lumping on a case by case basis, the details of which are summarised below.

Bombus:

We recorded at least 9 different species from the genus *Bombus*. Of these, 4 could be identified to species in the field. We confirmed the presence of *Bombus lucorum, B. cryptarum,* and *Bombus terrestris* from a small number of specimens that were taken from the site. However, the workers of these species are difficult to separate (Falk, 2015) and so in the same manner as other studies (see for example Goulson *et al.*, 1998), we had to lump them as a single morphotype: '*Bombus lucorum/terrestris'.* One bumblebee individual could not be identified and so is listed as *Bombus* sp. We recorded 4 cuckoo bumblebees (now *Bombus* but previously classed as *Psithyrus*) and lumped them as *Bombus (Psithyrus)* spp.

Lasioglossum:

From 51 specimens of the genus *Lasioglossum,* 44 were identified (7 species present). Four specimens were not possible to identify. The 7 identified species of *Lasioglossum* are not lumped, those not identified are lumped as *Lasioglossum* spp.

Tenthredo:

There were 4 specimens from the genus *Tenthredo,* 2 of which were identified as *Tenthredo arcuata* but the other two were not identifiable. All four are lumped as *Tenthredo* spp.

Halictus:

There were 13 specimens from the genus *Halictus,* 10 of which were identified as *Halictus tumulorum* but the other three were not identifiable. All 13 were lumped as *Halictus* spp.

Platycheirus:

There were 38 specimens from the genus *Platycheirus,* 11 of which were identified as *Platycheirus albimanus,* 2 as *P. pelatus* and 1 as *P. manicatus*. The remaining 24 were not identifiable and so all 38 were lumped as *Platycheirus* spp.

Sphaerophoria:

There were 46 specimens from the genus *Sphaerophoria.* Males for this genus were all identified; 10 as *Sphaerophoria interrupta,* 4 as *S. scripta* and 2 as *S. taenidata*. The 30 female specimens could not be identified so all 46 specimens were lumped as *Sphaerophoria* spp.

Eurithia:

There were 8 specimens from the genus *Eurithia,* 3 of which were identified as *Eurithia consobrina* and 1 as *S. anthophila.* The remaining 4 were not identifiable so all 8 were lumped as *Eurithia* spp.

Other:

There were 2 specimens from the super-family Ichneumonoidea, 2 from the family Tachinidae and 1 from the order Lepidoptera, that could not be identified. These are grouped under the label 'gs.sp.'.

4.3 Overview of the data

In total, 1468 individual interactions were recorded during the survey period. We aimed to collect a similar number of interactions in each survey so that the data set for each survey would be a similar size. Periods of rain during the first day of the S3 survey mean that the number of interactions recorded ($I = 93$) was lower than hoped. Therefore, we decided to survey for another 2 days so that the total number of interactions ($I = 325$) would be comparable to S1 ($I = 278$) and S2 ($I = 413$). The number of interactions recorded during each survey, and on each day, are summarised in Figure 4.7.

Twenty-six flowering plant species were identified at the site, 5 of which were grouped as described in section 4.2.c above. All plant species were present in at least one interaction survey and therefore part of the plant-pollinator network community.

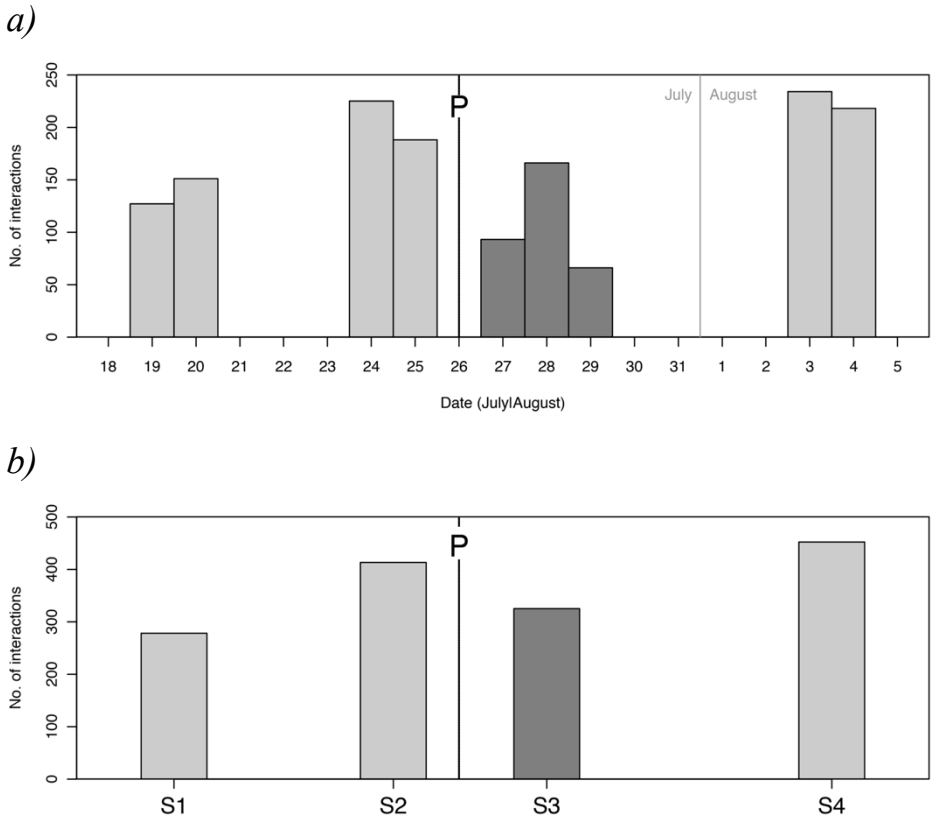


Figure 4.7 The number of interactions recorded presented ***a)*** *in detail and* ***b)*** *as a simple schematic. In* ***a)*** *we present the number of interactions recorded in each survey (S1-S4) broken down by days. The perturbation, on the 26th of July is indicated by the black line labelled P and the S3 survey is highlighted in darker grey. In the schematic* ***b)*** *we present the total number of interactions for each survey (S1-S4) with the gaps between the surveys on the x-axis representative of the time between the surveys. The perturbation is again indicated by the black line labelled P and the S3 survey is highlighted in darker grey.*

4.3.a The full survey networks F1-F4

Using the interaction data recorded for each survey, we produced 4 plant-pollinator networks. These networks depict all of the interactions recorded during each survey; they are the 'full' networks and so we call them F1, F2, F3 and F4. Each node in these networks represents a plant or pollinator species (or grouped species); note that we use the terms pollinator node and pollinator species interchangeably when referring to the network data for the remainder of this chapter. The number of pollinator nodes (*A*) and the number of plant nodes (*P*) are summarised in Table 4.2. Edges are weighted by the number of interactions observed. We present the F1, F2, F3 and F4 networks below (Figure 4.8, Figure 4.9, Figure 4.10 and Figure 4.11 respectively)

Table 4.1 A summary of the plant species recorded in the plant-pollinator network surveys S1-S4.

Code	Common name	Genus	Species	Grouped
BC	Buttercup	*Ranunculus*	spp.	*R. acris, R. bulbosos, R. repens*
SJ	Hairy St. Johns wort	*Hypericum*	*hirsutum*	-
MM	Musk mallow	*Malva*	*moschata*	-
BB	Bramble	*Rubus*	*fruticosus*	-
RC	Red clover	*Trifolium*	*pratense*	-
BT	Birdsfoot trefoil	*Lotus*	*corniculatus*	-
EN	Enchanters nightshade	*Circaea*	*lutetiana*	-
UH	Upright hedge parsley	*Torilis*	*japonica*	-
PN	Pignut	*Conopodium*	*majus*	-
HW	Hogweed	*Heracleum*	*sphondylium*	-
WW	Hedge Woundwort	*Stachys*	*sylvatica*	-
SH	Selfheal	*Prunella*	*vulgaris*	-
RW	Ragwort	*Senecio*	*jacobaea*	-
DY	Daisy	*Bellis*	*perennis*	-
CT	Creeping thistle	*Cirsium*	*arvense*	-
ST	Spear thistle	*Cirsium*	*vulgare*	-
MT	Marsh thistle	*Cirsium*	*palustre*	-
WT	Woolly thistle	*Cirsium*	*eriophorum*	-
DL	Dandelion	*Taraxacum*	*officinale*	*Micro species aggregate*
CE	Catsear	*Hypochaeris*	*radicata*	-
RH	Rough hawkbit	*Leontodon*	*hispidus*	-
HB	Hawksbeard	*Crepis*	spp.	*C. capillaris C. biennis*
PS	Perennial Sowthistle	*Sonchus*	*arvensis*	-

Table 4.2 Summary of the number of nodes and interactions in the 4 networks F1-F4 and the corresponding surveys S1-S4.

Survey	Network	No. of pollinator nodes (*A*)	No. of plant nodes (*P*)	No. of interactions (*I*)
S1	F1	54	19	278
S2	F2	53	20	413
S3	F3	58	15	325
S4	F4	65	21	452

In some analyses, we removed *C. eriophorum* from the F1, F2 and F4 networks in order to compare them to the F3 network (where there is no *C. eriophorum* because we removed it from the site). We do this by simply removing the *C. eriophorum* node and any interactions that involve *C. eriophorum* from F1, F2 and F4. We call these networks W1, W2 and W4 as they are without *C. eriophorum* (we also refer to the W3 network which is identical to F3).

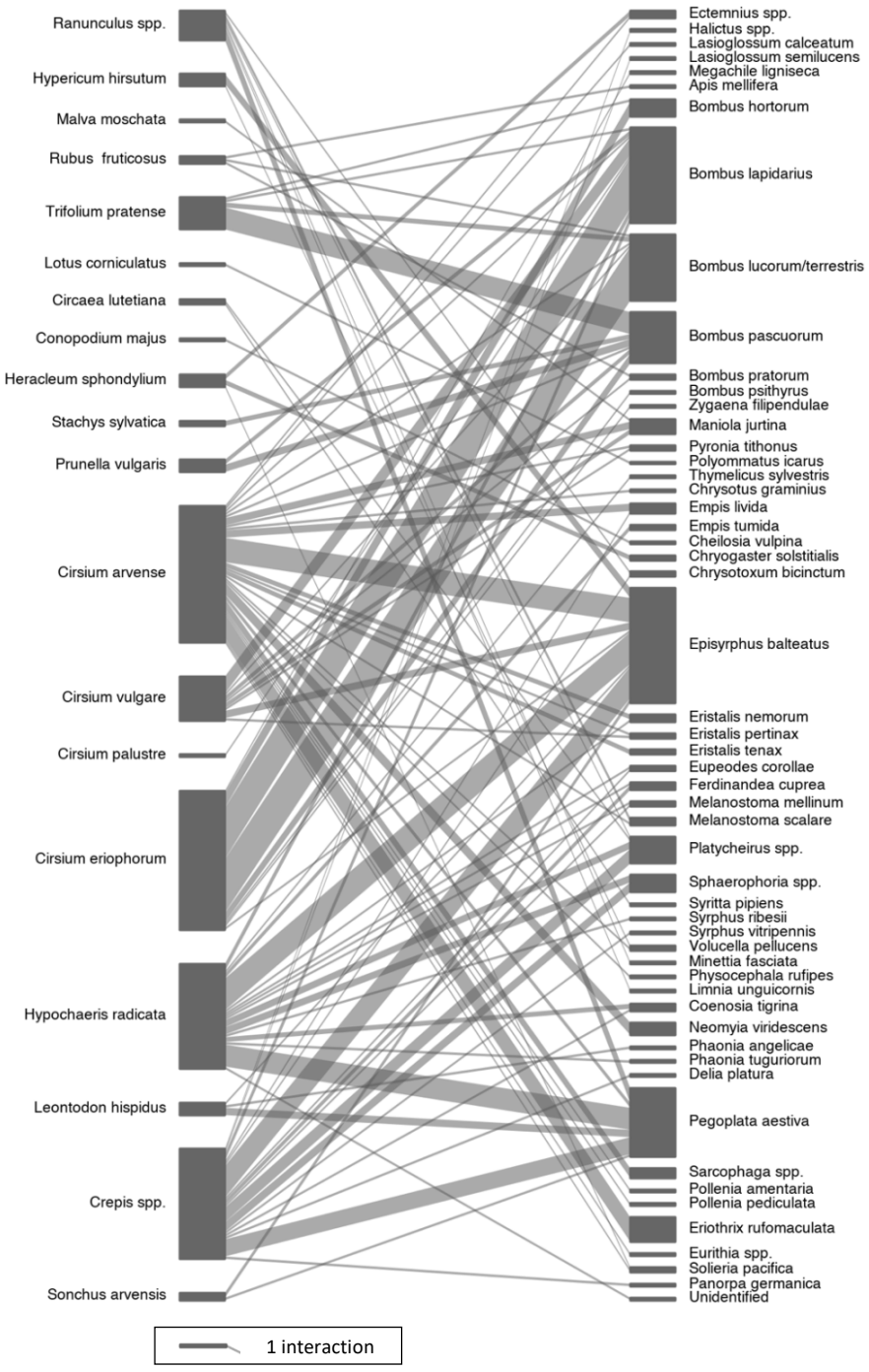


Figure 4.8 The F1 network showing the interactions recorded between all plants (left had side) and all pollinators (right hand side) from the S1 survey. In this and the following 4 figures (4.9 to 4.11): the height of each grey box represents the number of interactions recorded for that species. The key in the lower left-hand corner shows the scale for 1 interaction. The width of each interaction (grey line) is roughly representative of the number of interactions shared between the two species it connects.

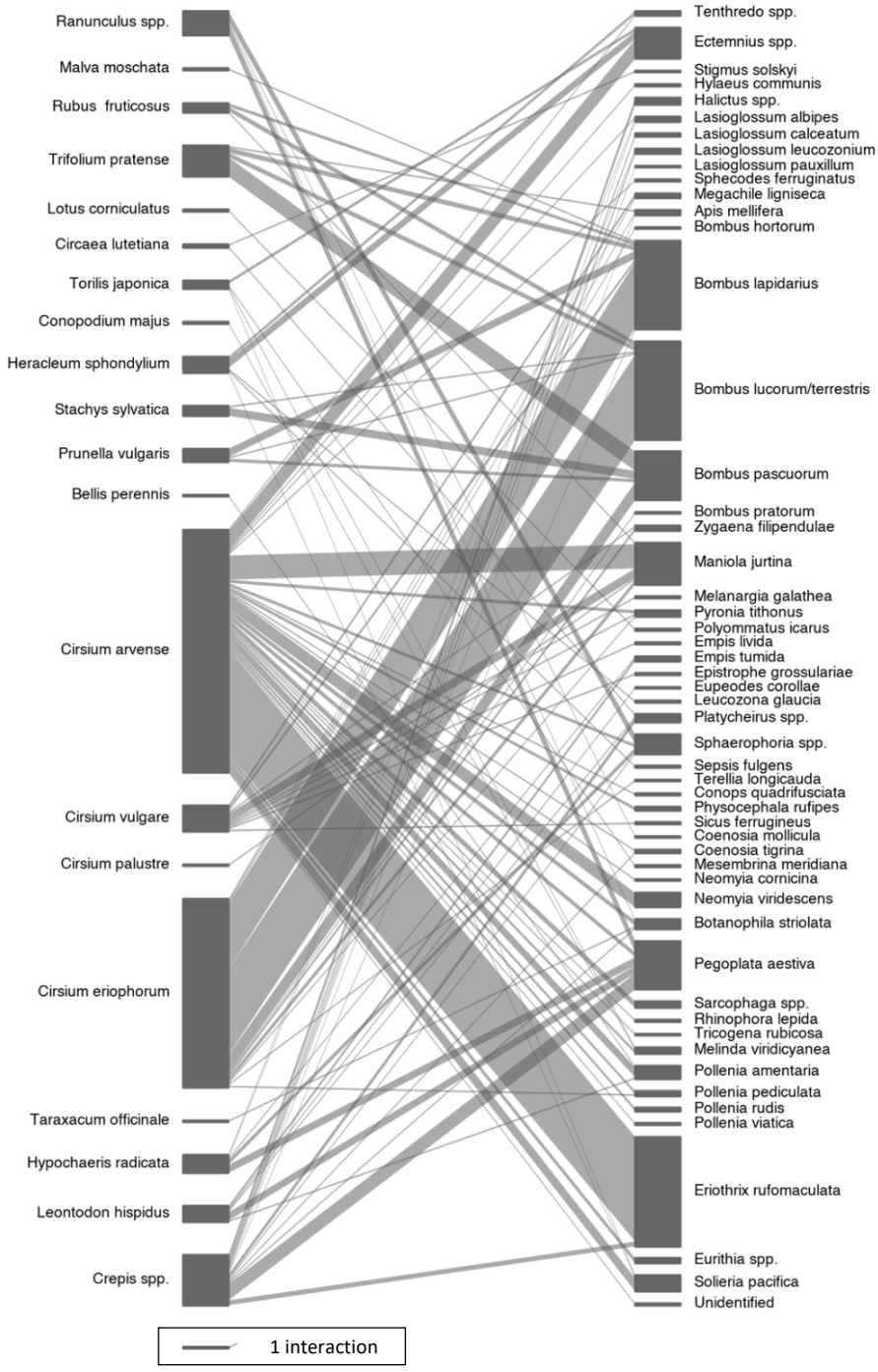


Figure 4.9 The F2 network showing the interactions recorded between all plants (left had side) and all pollinators (right hand side) from the S2 survey.

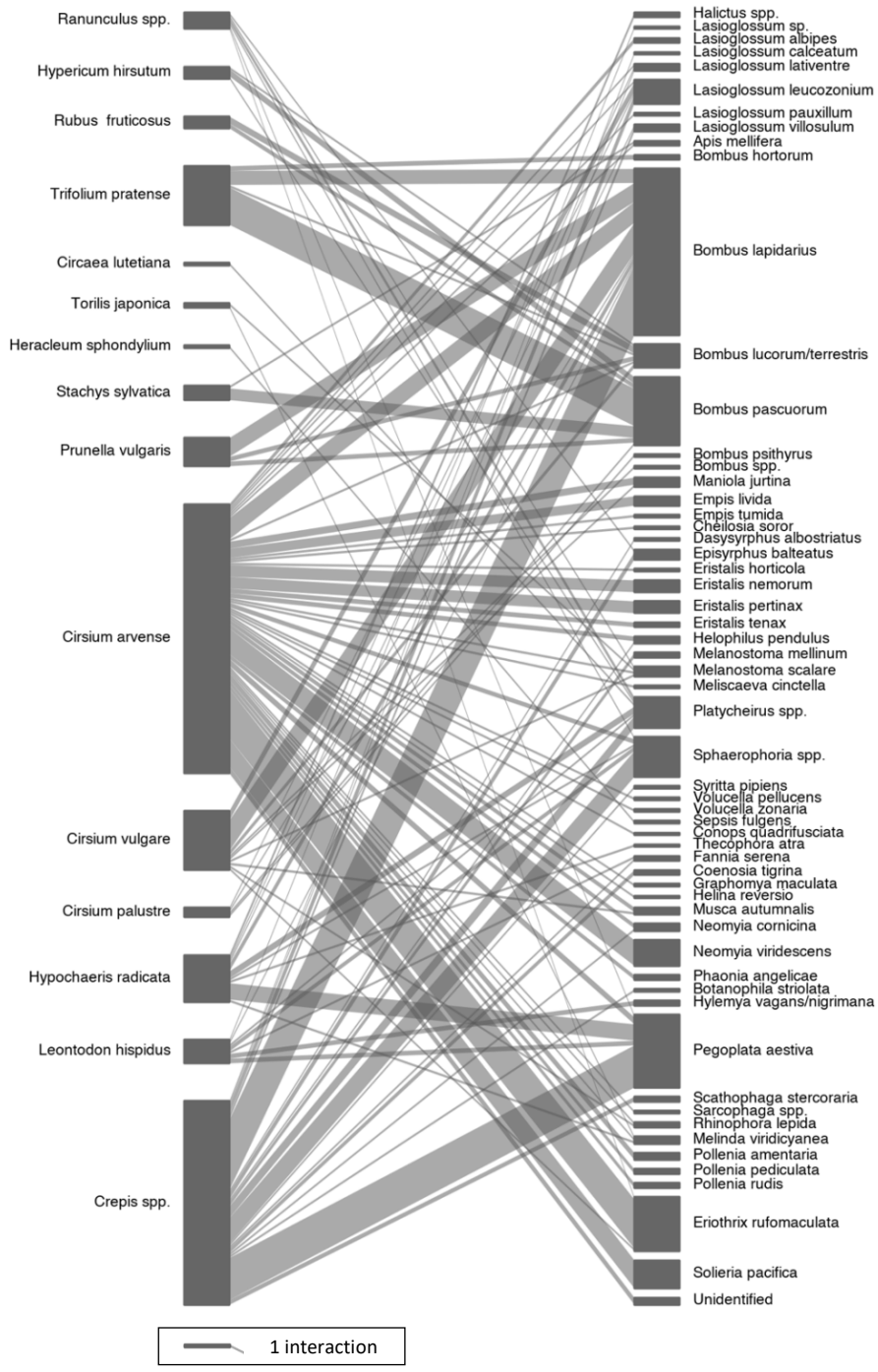


Figure 4.10 The F3 network showing the interactions recorded between all plants (left had side) and all pollinators (right hand side) from the S3 survey.

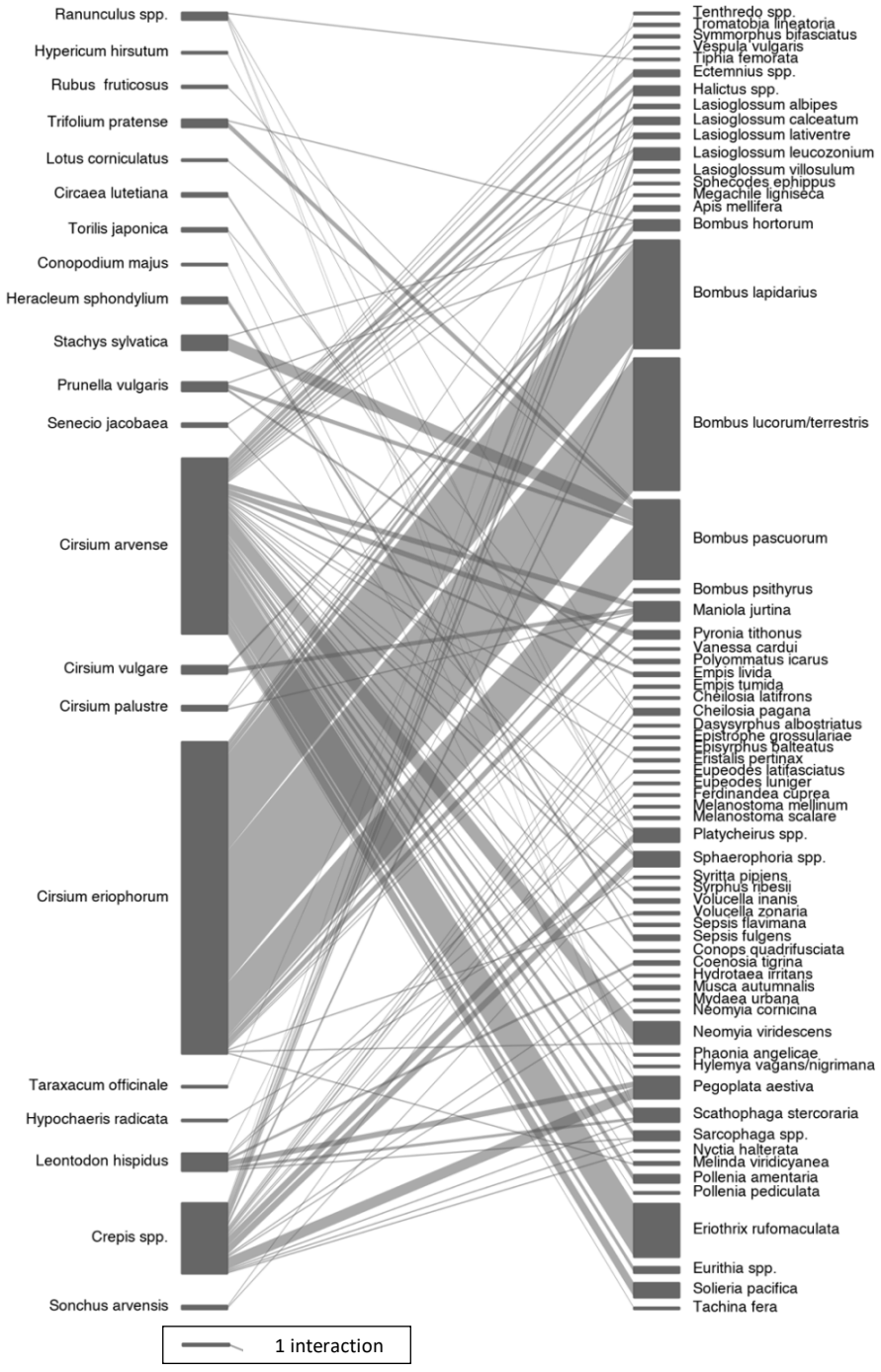


Figure 4.11 The F4 network showing the interactions recorded between all plants (left had side) and all pollinators (right hand side) from the S4 survey.

4.3.b The reduced networks R1-R4

When collecting data to describe a plant-pollinator network, we must take into account the potential effects of rare observations. Species that are rare within the survey site, and plant-pollinator interactions that are rare during the survey period, are more likely to be missed whilst sampling and therefore result in false negatives: *i.e.* edge(s) missing from the network. On the other hand, species or interactions that are common are less likely to be missed and are therefore the interactions observed are more reliable. This discrepancy in the reliability of network data can affect some types of analysis. Therefore, we created a set of reduced networks called R1, R2, R3 and R4 that are a chosen subset of the F1, F2 F3 and F4 networks. This subset only includes pollinator species that were observed a minimum of 10 times in at least one of the surveys (S1-S4), giving us $A = 13$. From the plant species that interact with these 13 plant species, we include only those that have a minimum of 3 interactions (with one or more pollinator species) in at least 1 survey, resulting in $P = 16$. These reduced networks (R1-R4) are presented in Figure 4.12 and Figure 4.13 and used in later analysis of indirect effects of the pulse perturbation.

Table 4.3 An overview of the three sets of networks used in this chapter.

Network prefix	Description	Comments
F	The full networks, containing all of the interactions recorded in the S1-S4 surveys	
W	The full networks but with all records for *C. eriophorum* removed	W3 is identical to F3
R	The well-sampled subset of the F networks	Insect nodes retained if they have a minimum of 10 visits recorded in the any of S1-S4. Plant nodes retained if they have a minimum of 3 interactions with the remaining subset of pollinator nodes.

Figure 4.12 The R1 network ***(a)****, and R2 network* ***(b),*** *showing the interactions between the plant species (left hand side/lower) and pollinator species (right hand side/upper) from the reduced species subset of the S1 and S2 surveys.*

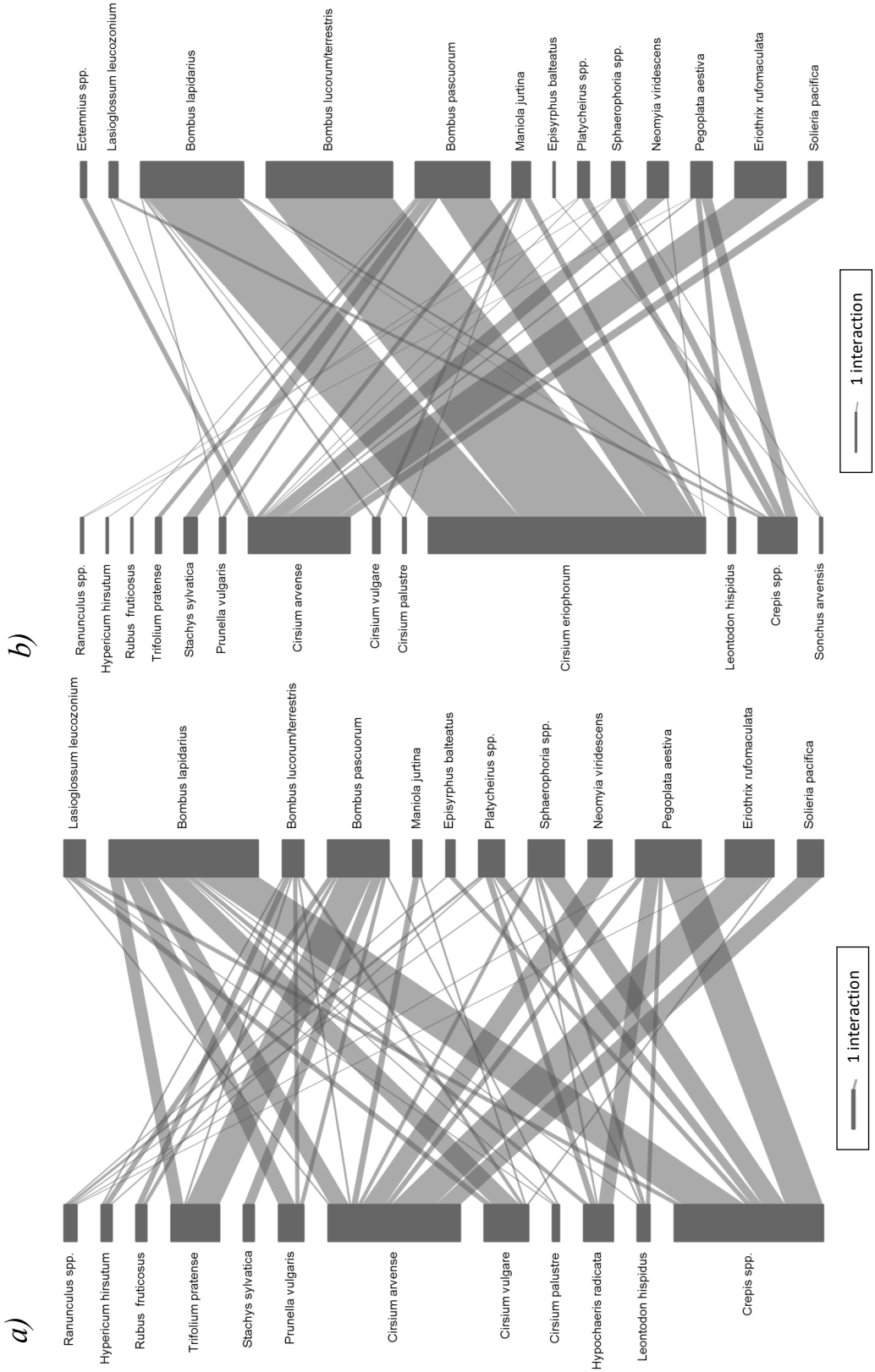


Figure 4.13 The R3 network ***(a)****, and R4 network* ***(b),*** *showing the interactions between the plant species (left hand side/lower) and pollinator species (right hand side/upper) from the reduced species subset of the S3 and S4 surveys.*

4.3.c Bumblebee populations at the site

Bumblebee species were feeding on *C. eriophorum* comparably high numbers (compared to other insect species) which is why we focused on them for examining the direct effect of the pulse perturbation. Because bumblebee individuals were marked and released when surveyed, we were able to estimate the population sizes of each species. By population size we mean the number of individuals present and feeding at the site, not necessarily the size of the colony/ies living at the site. From the population size estimates, and the number of bumblebees recaptured across surveys we can assess whether it is possible that the same bumblebee individuals are present throughout the survey period or that there is a high turnover of individuals between surveys. From records of how many bumblebees were marked, and how many were re-captured we were able to carry out a rough mark-release-recapture analysis to estimate the population size. Each survey (S1-S4) is made up of 2 or 3 survey days. We used the data from each survey day to estimate population size of the three most abundant bumblebee species (*Bombus lapidarius, B. pascuorum* and *B. lucorum/terrestris*) in each survey e.g. the first and second survey day were used to determine the population size during S1. We had to take into account two limitations of the data. Firstly, all bumblebees were released immediately after capture and did not have unique individual markings i.e. a bumblebee caught already with a mark could be re-caught a second time and we would not be able to distinguish this. Secondly, bumblebees caught in S1 and S2 were marked using the same colour and are therefore a re-catch on one of the S2 survey days could be from S1. To account for these limitations, we estimated the minimum and maximum population size, both using the Chapman Estimator (Sutherland, 2006). For the minimum population size estimate we used the total number of recaptures recorded based on the assumption that all recaptures were separate bumblebees. For the maximum population size estimate we set the number of recaptures to 1 based on the assumption that one individual was recaptured multiple times.

Our population estimates suggest that the number of bumblebees at the site during each survey increased from S1 and S4 (Figure 4.14). This might be a true increase or an artefact of increased sampling efficiency. Overall, we can deduce that there were hundreds of bumblebees at the site throughout the survey period. Records of re-catches (individuals that were caught with a mark already on them) indicate that some *B. lapidarius* individuals were present in all 4 surveys (Table 4.4). Similarly, re-catches show that *B. lucorum/terrestris* and *B. pascuorum* individuals were present in both S1/S2 and S3, and in S3 and S4 (Table 4.4). The population estimates are large enough that we would not expect to re-catch a large proportion of individuals and therefore the unmarked bees surveyed after

the removal and the regrowth of *C. eriophorum* were not necessarily new individuals that weren't present at the site before. The fact that re-catches did occur across surveys suggest that at least some of the same individuals were present throughout the whole survey period. Crucially the re-catch data indicates that there was not complete turnover of bumblebee individuals at the site over the whole survey period.

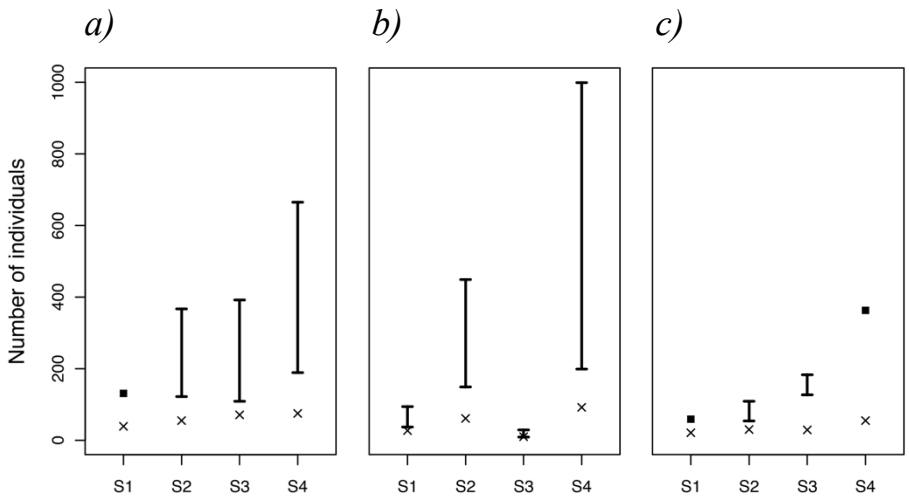


Figure 4.14 Population estimates of the three most abundant bumblebee species: ***a)*** *Bombus lapidarius,* ***b)*** *Bombus lucorum/terrestris and* ***c)*** *Bombus pascuorum. Vertical lines indicate the estimated range based on the max and min Chapman estimator values (max = re-catches are treated as one individual, min = re-catches are treated as unique individuals). Square points indicate Chapman estimator value where only one re-catch was recorded i.e. max = min. Crosses indicate the actual number of bumblebee individuals caught.*

Table 4.4 Re-catches of the 3 most abundant bumblebee species i.e. the number of individual bumblebees that were caught in multiple surveys according to markings.

Surveys	*B. lapidarius*	*B. luc/ter*	*B. pascuorum*
S1/S2 and S3	9	1	2
S3 and S4	3	2	1
S1/S2 and S4	0	0	0
S1/S2 and S3 and S4	3	0	0

4.4 Analysis methods

There are four main aspects to our analysis. First, we determine whether the network changed as a result of the removal of *C. eriophorum*. Secondly, we ask how the networks changed, by comparing standard network metrics across the 4 surveys and analysing the beta diversity of the networks across surveys. Thirdly, we assess the direct effects of the pulse perturbation on three abundant bumblebee species. Finally, we assess evidence of indirect effects on plant and pollinator species due to *C. eriophorum* removal.

4.4.a Perturbation and healing of the network

We expected that the removal of *C. eriophorum* would cause changes in the foraging patterns of pollinator species, which would be realised as changes in the interactions observed and consequently changes in the network. We expected that once *C. eriophorum* was allowed to re-flower, pollinators, and consequently observed interactions would return to their pre-perturbation state *i.e.* the network would 'heal'. The F2, F3 and F4 networks represent the interactions in the three predicted states: before, perturbed and healed. We expected that the F2 and F4 (before and healed) networks would be more similar than the F2 and F3 networks (before and perturbed) and the F3 and F4 networks (perturbed and healed). In other words, the correlation between F2 and F4 would be significantly greater than both the correlation between F2 and F3 and the correlation between F3 and F4. We expected that the removal of *C. eriophorum* would impact the structure of the network beyond, not just in terms of interactions involving *C. eriophorum*. We tested this using the W networks (where *C. eriophorum* is not included). We expected that the correlation between W2 and W4 would be significantly greater than both the correlation between W2 and W3 and the correlation between W3 and W4, as with the F networks.

To determine the similarity between the two networks in each pair (F2:F3, F2:F4 and F3:F4), we calculated the Pearson product-moment correlation coefficient (PPMCC) between the corresponding entries in the given two matrices. This gives us an observed r value, between 1 and -1, for each of the three pairs of networks ($r_{\mathrm{F2:F3}}$, $r_{\mathrm{F2:F4}}$ and $r_{\mathrm{F3:F4}}$). When $r = 1$, there is perfect positive linear correlation, if $r = -1$ there is perfect negative linear correlation and if $r = 0$ means there is no linear correlation. We expected that $r_{\mathrm{F2:F4}} > r_{\mathrm{F2:F3}}$ and $r_{\mathrm{F2:F4}} > r_{\mathrm{F3:F4}}$. We expected that the correlation between W2 and W4 would be significantly greater than both the correlation between W2 and W3 and the correlation between W3 and W4, as with the F networks. We used the same method to

determine the similarity of the W networks as with the F networks and our expectation is $r_{\mathrm{W2:W4}} > r_{\mathrm{W2:W3}}$ and $r_{\mathrm{W2:W4}} > r_{\mathrm{W3:W4}}$.

In order to determine if the differences between our observed r values are significant we used a network randomisation protocol. Using $r_{\mathrm{F2:F4}} > r_{\mathrm{F2:F3}}$ as an example, our method was as follows. We took the network that appears in both r values (F2 in this example) and added one to all entries in the F2 matrix so that there were no 0s. Then we calculated the proportion of interactions in each entry of the matrix as a fraction of the total number of interactions in the matrix to give us a probability matrix. We generated a null network with the probability of an interaction occurring between a plant (p_i) and pollinator (a_j) equal to proportion of interactions between p_i and a_j in the probability matrix. By changing 0 entries to 1 we are assuming that an interaction between the two species in question is rare (we did not observe it) but not impossible. We then calculated $r_{null:\mathrm{F4}}$ and $r_{null:\mathrm{F3}}$ using the PPMCC. We repeated this to generate 9,999 null networks and the corresponding $r_{null:\mathrm{F4}}$ and $r_{null:\mathrm{F3}}$ values. We calculated the difference between each $r_{null:\mathrm{F4}}$ and $r_{null:\mathrm{F3}}$ to get a distribution of null differences. From this we determined if the observed difference between $r_{\mathrm{F2:F4}} > r_{\mathrm{F2:F3}}$ was significant and not within the expectation of difference based on variation in sampling as modelled in our randomisation protocol. We used this randomisation protocol to test the significance of the difference between the pairs of networks (F and W) as stated in the expectations above.

4.4.b Changes to the structure of the network as a result of the pulse perturbation

Having determined that the network structure did change, we ask how it changed. We calculated several standard network metrics, often used by network ecologists. In the spirit of Brosi, Niezgoda and Briggs (2017) who studied the effect of removing key bumblebee species on network structure, we calculated the following metrics for S1, S2, S3 and S4 as well as W1, W2, W3 and W4, using the 'networklevel' function from the package 'bipartite' (Dormann, Gruber and Fruend, 2008) in R (R Core Team, 2017):

- total number of plant species visited: *P*
- total number of pollinator species observed: *A*
- mean species-level specialization: d′
- connectance: c
- resource overlap: niche.overlap.HL
- niche partitioning/complementarity: C. score

- nestedness: network temperature

Mean species-level specialization (d′) calculates the mean specialisation index (based on Shannon's diversity index) where 0 is no specialisation and 1 is perfect specialisation of a given species (Blüthgen, Menzel and Blüthgen, 2006). Connectance is the number of observed interactions as a fraction of total number of possible interactions in a binary network (Martinez, 1992). Resource overlap (niche.overlap.HL) quantifies the amount of competition between pollinator species caused by pollinators visiting the same subset of plant species (based on the Horn index from Krebs, 1999). Niche partitioning/complementarity (C. score) is similar to resource overlap but quantifies the amount of differentiation of niches between species indicating competition avoidance (Stone and Roberts, 1990). Nestedness (network 'temperature') in a plant-pollinator network refers to the degree to which species with few interactions have a subset of the interactions of species with more interactions (Rodriguez-Girones and Santamaria, 2006). See Dormann *et al.* (2009) and Delmas *et al.* (2018) for an overview of these and other network metrics. We converted the network to binary form where the metric calculation required it to be so. We used Grubb's outlier test to determine if the observed value for each metric in F3 was a significant outlier (Grubbs, 1969).

In addition to these standard network metrics, we calculated the beta-diversity i.e. 'temporal turnover', of the networks. This quantifies the amount of change between two networks partitioned into the turnover of interactions and species (Koleff, Gaston and Lennon, 2003; Poisot *et al.*, 2012). We expected that in our networks, pollinator and plant turnover would be relatively low, but interaction turnover would be high between S2:S3 and S3:S4 as species reorganised as a result of the loss and return of *C. eriophorum.* Using the same method as Kemp *et al.*, (2017) we calculated the total beta diversity (Jaccard index: B_{cc}) and the different components responsible for interaction turnover (Novotny, 2009):

B_p = turnover of interaction due to the absence of the plant species from one network,

B_a = turnover of interaction due to the absence of the pollinator species from one network,

B_{pa} = turnover of interaction due to the absence of both plant and pollinator species in one network,

B_0 = both plant and pollinator species are present in both networks, but the interaction is absent in one.

These are summarised in Table 4.5, along with a description of the ecological hypothesis behind each component. We calculate B_{cc} and its components for each pairwise combination of the 4 observed networks (F1:F2, F2:F3, F4:F4, F2:F3 etc) in binary form in order to determine if the differences in the networks are mainly a result of changes in interactions or a result of changes in pollinator species at the site. We use binary versions of the matrices because this is the standard method and although it is possible to calculate beta diversity from weighted interaction data, there are known issues with interpretation of weighted results (Barwell, Isaac and Kunin, 2015). One issue we anticipated with using the binary data is that rarer interactions would have the same influence as those which we know from the weighted data to be much more common resulting in an overestimated turnover. In order to address this, we also carried out this analysis on reduced versions of the networks; R1, R2, R3 and R4 (see section 4.3.b).

Table 4.5 The total interaction turnover B_{cc} can be partitioned into 4 components, described here. If one component makes up the majority of the observed turnover, then ecological hypothesis can be applied to explain the ecological mechanisms behind the observed network structure. Table adapted from Novotny (2009).

Component	Definition	Ecological hypothesis
$\boldsymbol{B_{pa}}$	Turnover of interaction due to the absence of both plant and pollinator species in one network	**Specialization** between plants and pollinators. When a plant is no longer a viable food source, pollinators leave to forage at an alternative site, or die.
$\boldsymbol{B_a}$	Turnover of interaction due to the absence of the pollinator species from one network	Pollinators are lost from the network due to limited lifespan or because they move between foraging sites.
$\boldsymbol{B_p}$	Turnover of interaction due to the absence of the plant species from one network	Plants are no longer a viable food source (e.g. past peak nectar production) and so pollinators switch to alternative plants.
$\boldsymbol{B_0}$	Both plant and pollinator species are present in both networks, but the interaction is absent from one	**Generalisation** between plants and pollinators. Pollinators are able to visit a board range of plant species are so are observed switching through time.

4.4.c Direct effects of the pulse perturbation: response of bumblebee species

So far, we have focused on network level effects of the pulse perturbation. Each network was the result of sampling the observed interactions at the site and therefore a product of pollinator behaviour. In order to understand the observed changes in the networks, we examined direct and indirect effects of the removal of *C. eriophorum* on the pollinators in the survey.

Direct effects of the pulse perturbation affect pollinator species that interact directly with *C. eriophorum* at some point during the survey period. There were 18 pollinator species (nodes) that interacted with *C. eriophorum* in at least one survey. Most of these species had fewer than 15 interactions with *C. eriophorum* over the whole survey period (S1-S4). However, 3 species had many more; *Bombus lapidarius* (=167)*, Bombus pascuorum* (=129) and *Bombus lucorum/terrestris* (=52). This is unsurprising as we knew from the 2017 surveys of the site (Chapter 3) that *C. eriophorum* was a much-used resource for bumblebees. At least 10 interactions were recorded for each of these species in each survey. All three species also had a large proportion (>49%) of their interactions with *C. eriophorum* in S1, S2 and S4 combined. We focused our analysis of direct effects on the foraging patterns of the three bumblebee species in S2 and S3.

B. lapidarius, B. pascuorum and *B. lucorum/terrestris* all fed on *C. eriophorum* in S1 and S2. In S3 they could not feed on *C. eriophorum* as it had been removed from the site. In one sense, we know these species were affected by the pulse perturbation because they lost a major resource. We were interested in whether these species changed their foraging patterns when *C. eriophorum* was not available. To do this we looked at the other plant species they were observed feeding on. We tested two alternative hypotheses for foraging patterns in S3 when *C. eriophorum* is not available: H_1 - the bumblebee species foraged on the same non-*C. eriophorum* species they visited in S2, in the same proportions but greater numbers, or H_2 - the bumblebee species foraged on new plant species to replace the gap left by the loss of *C. eriophorum,* as well as the same non-*C. eriophorum* species they visited in S2. We call these the expansion and replacement hypotheses respectively (see Figure 4.1 for visualisation).

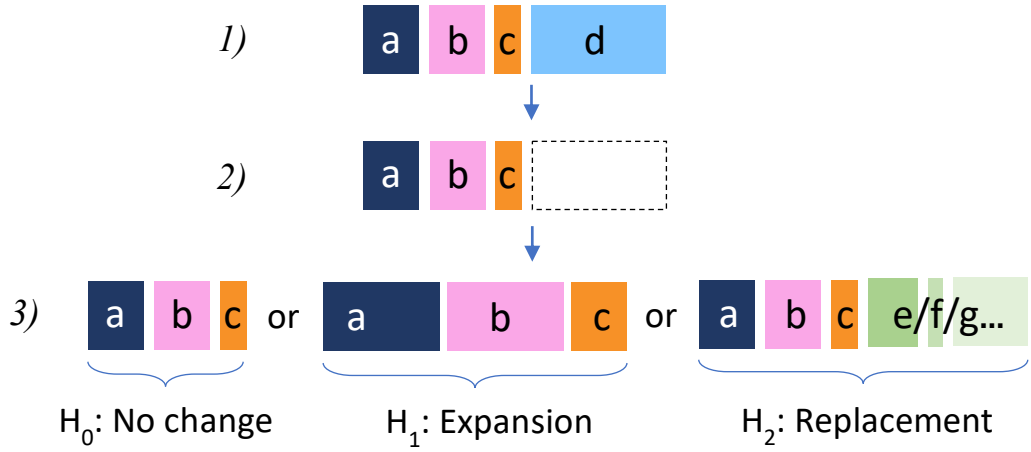


Figure 4.15 Visualisation of the expansion and replacement hypothesis. ***1)*** *shows the foraging pattern of a given insect species with the width of the bars representing the proportion of interactions with each plant species its visits (a-d). In* ***2)*** *the plant species 'd' is lost or removed as a foraging option – just like C. eriophorum is in our experiment. In step* ***3)*** *we see the three different responses. H_0: Null hypothesis, no change observed in plant species visited, indicating the loss of the insect individuals that were feeding on plant 'd' in step 1. H_1: Expansion – the pollinator expands its existing foraging options (a, b, and c), in the same proportions as before, to fulfil its foraging requirements. H_2: Replacement – the pollinator expands the range of flower species it feeds on (to one or more other species e.g. e/f/g) to fulfil the gap left by the loss of 'd'.*

We test these two alternative hypotheses, for each of the three, bumblebee species, by calculating the Chi-squared (χ^2) statistic to determine if the observed proportions of interactions (S3) is significantly different from the expected proportion of interactions (calculated from S2; the survey immediately preceding the *C. eriophorum* removal). For H_1 the expected proportions of interactions are simply the proportions of interactions with non-*C. eriophorum* plant species in S2. For H_2 our expectation is the proportions of interactions observed in S2 but with the proportion of visits to *C. eriophorum* taken as the proportion of visits to any other plant species. For each bumblebee species, we calculated the χ^2 statistic for each hypothesis. We used a randomisation model in order to determine if these χ^2 statistics are significant. For example; using the expected foraging proportions of *B. lapidarius* under H_1, we generated 99,999 null foraging patterns. For each null foraging pattern, we calculated the χ^2 statistic, resulting in a distribution of 9,999 null χ^2 statistics. From this distribution, we determine the p-value and significance of our observed χ^2.

4.4.e Indirect effects of the pulse perturbation: plants and pollinators

Indirect effects are those observed in species that are not directly connected to *C. eriophorum*. In order to quantify potential indirect effects, we determined the absolute

change in degree and the absolute change in the numbers of interactions between F2 and F3 for the 80 pollinator species and 22 plant species (excluding *C. eriophorum*) that were observed in S2. We did this by calculating the absolute change in the corresponding entries of the F2 and F3 matrices that describe the F2 and F3 networks (see Figure 4.16). Weighted matrices (weighted by number of interactions) were used to calculate change in interactions and binary matrices (binary for presence of absence of interactions) were used to calculate change in degree. This method accounts for a change in the species interacted with, even if the overall degree or number of interactions remains the same.

Example F2 matrix:

	A_1	A_2	A_3
P_1	1	0	2
P_2	0	5	10

Example F3 matrix:

	A_1	A_2	A_3
P_1	0	1	5
P_2	2	6	4

Absolute change in degree (F2 to F3):

	A_1	A_2	A_3
P_1	1	1	0
P_2	1	0	0

Absolute change in interactions (F2 to F3):

	A_1	A_2	A_3
P_1	1	1	3
P_2	2	1	6

Figure 4.16 Example of absolute change in degree and absolute change in interactions between an example F2 and F3 matrix. Note that a change in the species interacted with is accounted for in both measures of change e.g. the degree of A_1 is the same in F2 and F3 but the total absolute change in degree for A_1 is 2 (1+1) because the species interacted with changed.

We expected that if there were indirect effects that cascaded through the network as a result of the removal of *C. eriophorum*, they would be greatest in the species that were close to *C. eriophorum* in the network. We define species as being close to *C. eriophorum* in the network if they directly interact with *C. eriophorum* or a path can be traced from them to *C. eriophorum* via a small number of other species. Species that are further away from *C. eriophorum* are less likely to be influenced by the pulse perturbation. To quantify this we calculated the interaction weighted distance from *C. eriophorum* using the 'distance_w' function from tnet (Opsahl, 2015) in R (R Core Team, 2017) for each of the 80 pollinator species and 22 plant species (excluding *C. eriophorum*) in the F2 network. 'distance_w'

calculates the distance from one node to another, using path length (the shortest number of steps to get from node to another) and taking into account the weights of the interactions on that path. If there were measurable indirect effects, we expected that absolute change in degree and/or interactions would be a function of the weighted distance from *C. eriophorum* where nodes with a short distance will change the most, and nodes with a long distance will change the least. However, there were two other factors that we wished to take into account as they were likely to have confounding affects. These factors were the observed degree and observed number of interactions in F2. The potential confounding affects were twofold. Firstly, rare species were likely to have a low degree and/or number of interactions, are more likely to be further away from *C. eriophorum* in the network (due to fewer path options) and were less reliably sampled. For these species, there would be a greater chance of observing change which is a result of their rarity rather than indirect effects. Secondly, species that were common and abundant were likely to have a high degree and/or number of interactions and were more likely to be closer to *C. eriophorum* in the network due to many path options (though this is partially accounted for in the weighted distance metric). We had a greater chance of reliably sampling these species but if they were generalist it would be possible that we observed change in their degree/interactions as a result of their generalist behaviour rather than indirect effects of the pulse perturbation. Therefore, we calculated degree and total number of interactions observed in S2 (F2 network) for the 80 pollinators and 22 plant species.

In order to determine if there were knock on effects that spread out from *C. eriophorum,* we modelled the absolute change in degree and the absolute change in interactions using a generalised linear model (GLM) with independent variables: weighted distance from *C. eriophorum*, degree (in F2) and the number of interactions (in F2). We used separate models for plants and pollinators because the distribution of interactions and degrees are different in these two groups; plants tend to have higher degrees and higher numbers of interactions than pollinators. We assumed a quasi-Poisson distribution of residuals because our data were over-dispersed compared to a standard Poisson distribution. We scaled the degree in F2 and number of interactions in F2 by $\log_2$ because they are highly skewed and only use one of these variables in each model as they are highly correlated (see Figure 4.17). The dependent and independent variables used in our GLMs are summarised in Table 4.6. We expected that weighted distance would be a significant predictor of absolute change in our models, indicating that knock on effects spread through the network from *C. eriophorum*.

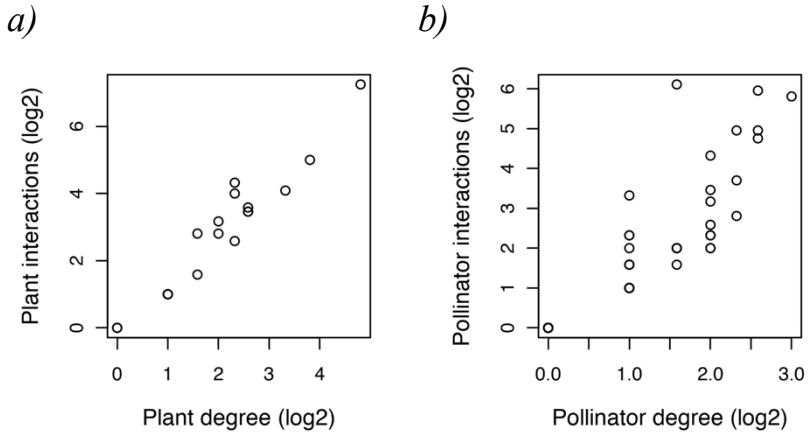


*Figure 4.17 Degree and number of interactions (from the F2 network) are colinear on a log2 scale for both **a)** plants and **b)** pollinators.*

Table 4.6 A summary of the dependent and independent variables in the GLMs used to test for knock-on effects of the pulse perturbation.

	Dependent Variable	Independent variables
Plants	Absolute change in degree	Weighted distance, log2(degree in F2)
	Absolute change in number of interactions	Weighted distance, log2(degree in F2)
Pollinators	Absolute change in degree	Weighted distance, log2(degree in F2)
	Absolute change in number of interactions	Weighted distance, log2(interactions in F2)

Having determined if there were knock on effects, we explored whether it was possible to predict the effect of the perturbation on the degree and number of interactions of plants and pollinators. To do this, we first calculated the degree and number of interactions (of each node) in F3 as a fraction of the degree and number of interactions in F2; we call these the degree ratio and interaction ratio. We then modelled both ratios, on a $\log_2$ scale as a function of weighted distance from *C. eriophorum.* As above, we treated plants and pollinators separately.

4.5 Results

4.5.a Changes to the structure of the network as a result of the pulse perturbation

We found that the network correlations (see Table 4.7) of the full (F) networks matched our expectation (Table 4.8); the before (F2) and healed (F4) networks were significantly more positively correlated to each other than to the perturbed network (F3). This indicates that the removal of *C. eriophorum* changed the network, and that after regrowth the network healed to be more similar to its pre-perturbation state. However, this was not the case for the W networks (*C. eriophorum* not included), (Table 4.7 and Table 4.8). The results of the randomisation protocol to test for significance are summarised in Figure 4.18. Overall, these results suggest that the before and after networks (F2 and F4) were more positively correlated to each other than the perturbed network (F3) because of the large difference in the number of interactions involving *C. eriophorum* and that there was not a significant change in the rest of the network that could not be separated from sampling variation.

Table 4.7 Summary of the r values calculated using the PPMCC between the before (2), perturbed (3) and healed (4) networks, both full (F2, F3 and F4) and with C. eriophorum removed (W2, W3 and W4).

Networks		r (PPMCC)	Networks		r (PPMCC)
F2	F3	0.405	W2	W3	0.554
F2	F4	0.859	W2	W4	0.885
F3	F4	0.224	W3	W4	0.626

Table 4.8 The results of the randomisation protocol to test for the significance of the differences between the network correlations according to our expectations. In all four cases, the observed network correlations were in keeping with our expectations. However, only the observations for the F networks were significant (*).

Expectation	Observed	Significance
$r_{F2:F4} > r_{F2:F3}$	$0.859 > 0.405$	$p = 0.025$ *
$r_{F2:F4} > r_{F3:F4}$	$0.859 > 0.224$	$p = 1 \times 10^{-4}$ *
$r_{W2:W4} > r_{W2:W3}$	$0.885 > 0.554$	$p = 0.279$
$r_{W2:W4} > r_{W3:W4}$	$0.885 > 0.626$	$p = 0.998$

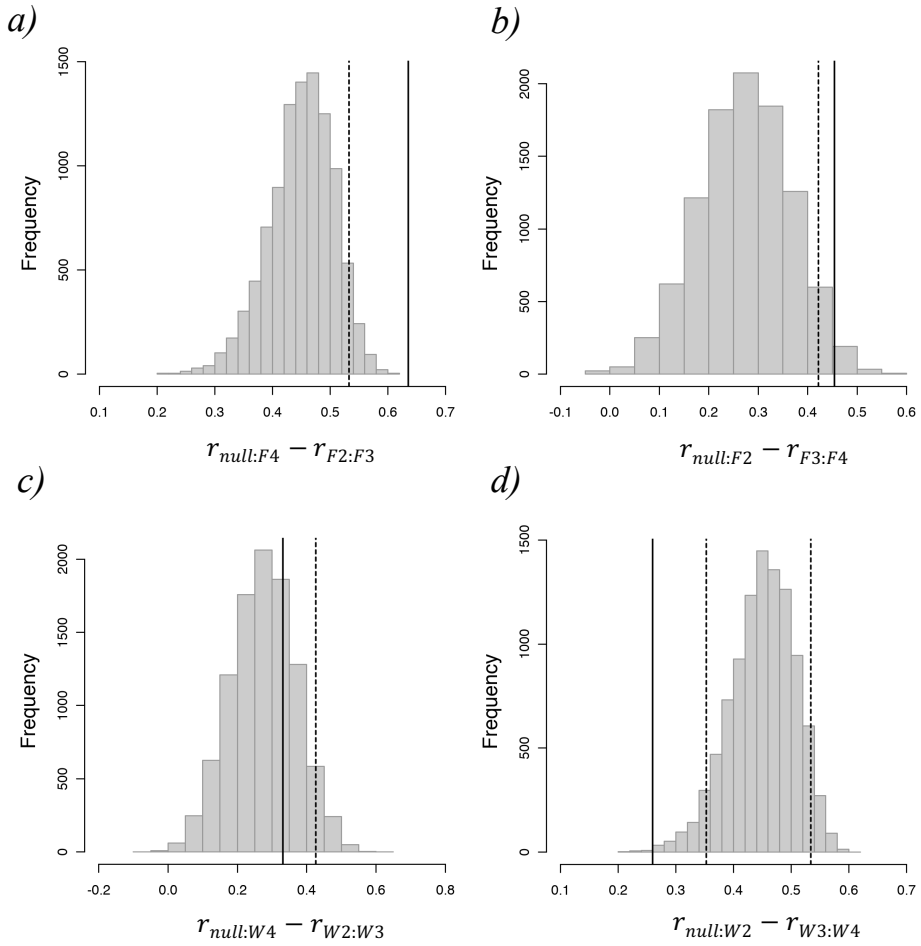


Figure 4.18 The distributions of difference in r from the randomisations protocol to test the significance of the observed network correlations. The observed difference in correlation (indicated by the solid vertical line) between the before (F2) and healed (F4) networks was significantly greater than the correlation between the ***a)*** *the before (F2) and perturbed (F3) networks and* ***b)*** *the perturbed (F3) and healed (F4) networks. The dashed vertical line indicates the difference at the 95% confidence limit. On the contrary, the observed difference in correlation between the before (W2) and healed (W4) networks without C. eriophorum was not significantly greater than the correlation between the* ***c)*** *the before (W2) and perturbed (W3) networks and* ***d)*** *the perturbed (W3) and healed (W4) networks. In* ***d)*** *the observed difference in correlation was significantly smaller; the observed value lies to the left of the 5% confidence limit indicated by the left-hand dashed line.*

4.5.b Changes to the structure of the network as a result of the pulse perturbation

Significantly fewer plant species were visited when *C. eriophorum* was removed from the site i.e. in S3 (*Grubbs test coefficient* = 1.976, $p < 0.05$) (Figure 4.19). Additionally, the decrease in plant species in F3 was not accompanied by a decrease in pollinator species. More pollinator species were observed in F3 and F4 than F1 and F2. This suggests that the loss of *C. eriophorum* did not, in general, cause pollinators to expand their foraging repertoire to other plant species previously not visited as might be expected. On the contrary,

this suggests that pollinator species are changing their foraging when *C. eriophorum* is removed to feed on a smaller number of plant species overall, though not necessarily as individuals.

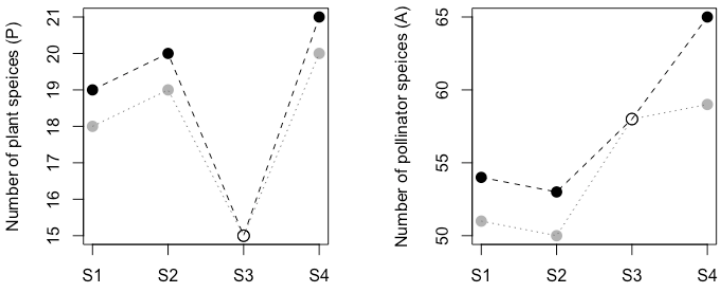


Figure 4.19 The number of plant (P) and pollinator (A) species observed. The black points correspond to the network in its observed form (F1-F4). The grey dots correspond to the W network where C. eriophorum has been removed from the network (W1-W4). The white points correspond to the observed F3 network where C. eriophorum has been removed in the field. Lines are plotted to guide the eye.

We found that mean pollinator specialisation was significantly lower (*Grubbs test coefficient* = 2.119, $p < 0.01$) for the observed F3/W3 network (Figure 4.20) compared to the other networks. In ecological terms this suggests that in S3, pollinator foraging behaviour was less specialised than in the other surveys. Pollinators were, on average feeding on a wider variety of available plant species, though considering that fewer plant species were visited (Figure 4.19) is it possible that there was greater overlap in the plant species visited by different pollinators. The observed F3/W3 network was not a significant outlier for any of the other network metrics, however patterns were observed that agreed with the significant changes in the corresponding network metrics found by Brosi, Niezgoda and Briggs, (2017) following their experimental removal of a pollinator species. We found that connectance appeared to increase in F3 compared to F1, F2 and F4, niche partitioning decreased in F3, and niche overlap increased in F3. In terms of nestedness, there is no observable difference in F3 compared to F1, F2 and F4. Nestedness appears most different in the observed F4 when it decreased (though not significantly, $p=0.391$). Interestingly the observed decrease in niche partitioning and increase in niche overlap both agree with our interpretation of the decrease in plant species visited (Figure 4.19) in F3 and the decrease in specialisation in F3 discussed above. These metrics in combination suggest that the removal of *C. eriophorum* caused an overall reduction in the number of plant species visited, but an increase in plant species visited by pollinator species, less specialisation and more competition.

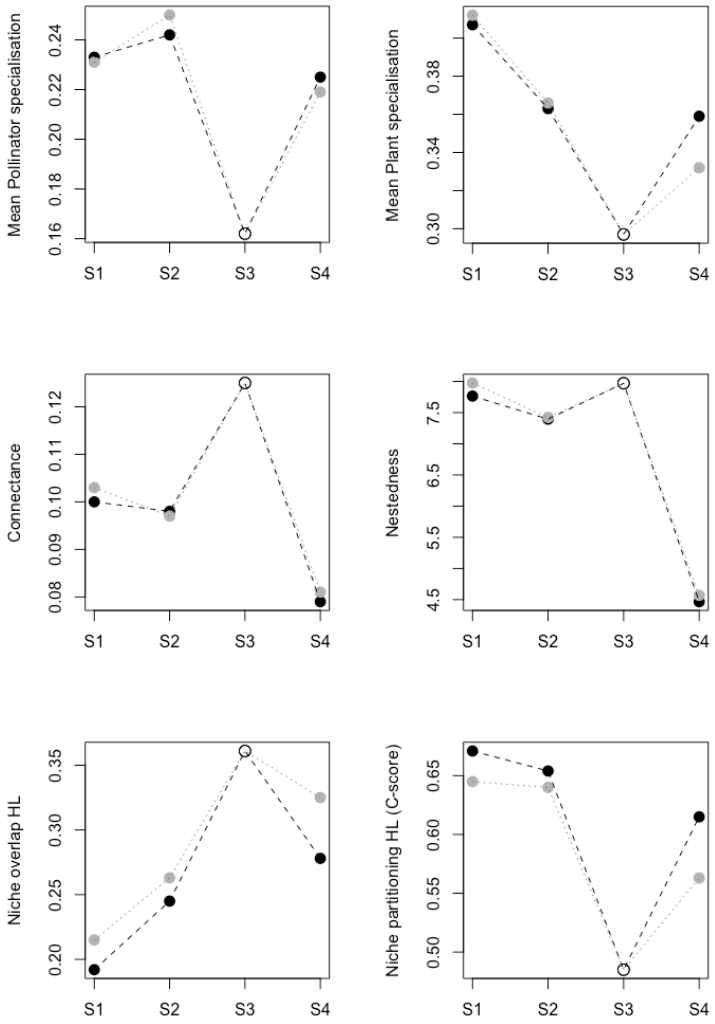


Figure 4.20 Network metrics for the 4 F networks (F1-F4). The black points correspond to the network in its observed form. The grey points correspond to the observed W networks C. eriophorum has been removed from the network (W1-W4). The white points correspond to the observed F3 network where C. eriophorum has been removed in the field.

Beta diversity (i.e. turnover), is high for all comparisons of the F networks (B_{cc} > 0.7), in line with previous studies on plant-pollinator network turnover (Carstensen *et al.*, 2014) and very little variation is seen (Figure 4.21). The only noticeable difference is a higher turnover between F1 and F4 compared to the other networks, which is logical considering these are the two networks furthest apart in time (13 days apart). In all cases, the largest components of B_{cc} are B_0 (interaction turnover) and B_a (pollinator turnover). This is in line with our expectation that the interaction and pollinator turnover will be higher compared to B_p and B_{pa} (plant and plant + pollinator) turnover. The results from the reduced networks

(R1-R4) give a clearer picture of the differences in turnover between different pairs of networks. Overall turnover is less in all cases (B_{cc} < 0.7) compared to the F networks, suggesting that the high turnover for the F networks is at least in part caused by rare species. For all of the R networks, interaction turnover was the largest component, greater than all other components combined. This indicates that there was a high level of generalism, where pollinators switched to feed on different plant species over time, despite the plant species remaining present and fed on by other species. Notice also that overall turnover was smallest between R2 and R4 (B_{cc} = 0.47) and there was no plant species turnover (B_p = 0). R2 and R4 are most similar in terms of species composition and interactions, despite being separated by 8 days, and the perturbation. This indicates 'healing' of the network to its pre-perturbation state once *C. eriophorum* has re-flowered.

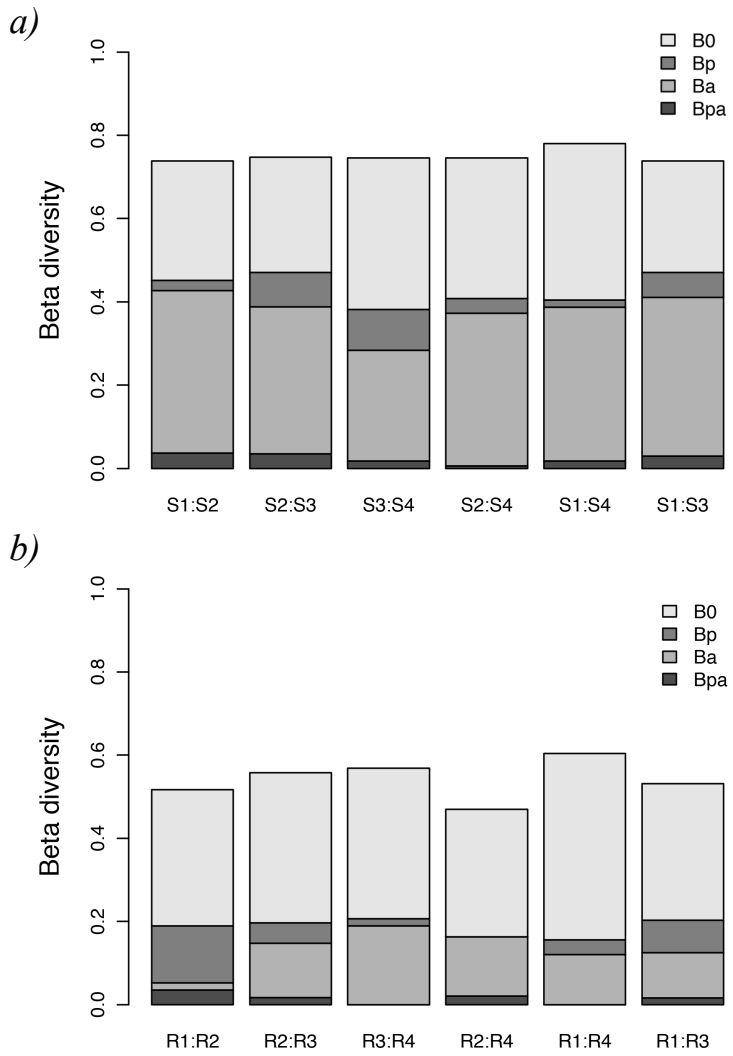


Figure 4.21 Beta diversity between each pair of networks, broken down in to the different components that contribute to turnover: B_o = Interaction turnover, B_p = Plant turnover, B_a = Pollinator turnover, B_{pa} = plant and pollinator turnover. ***a)*** *Beta diversity calculated on the whole observed networks.* ***b)*** *Beta diversity calculated on the reduced networks.*

4.5.c Direct effects of the pulse perturbation: response of bumblebee species

We found that the three most abundant bumblebee species, *Bombus lapidarius, B. lucorum/terrestris* and *B. pascuorum* had distinct and different reactions to the removal of *C. eriophorum*. To the best of our knowledge, we are the first to show with experimental data that different bumblebee species have different responses to the loss of a major foraging resource. These results are presented in Figure 4.22 and Figure 4.23.

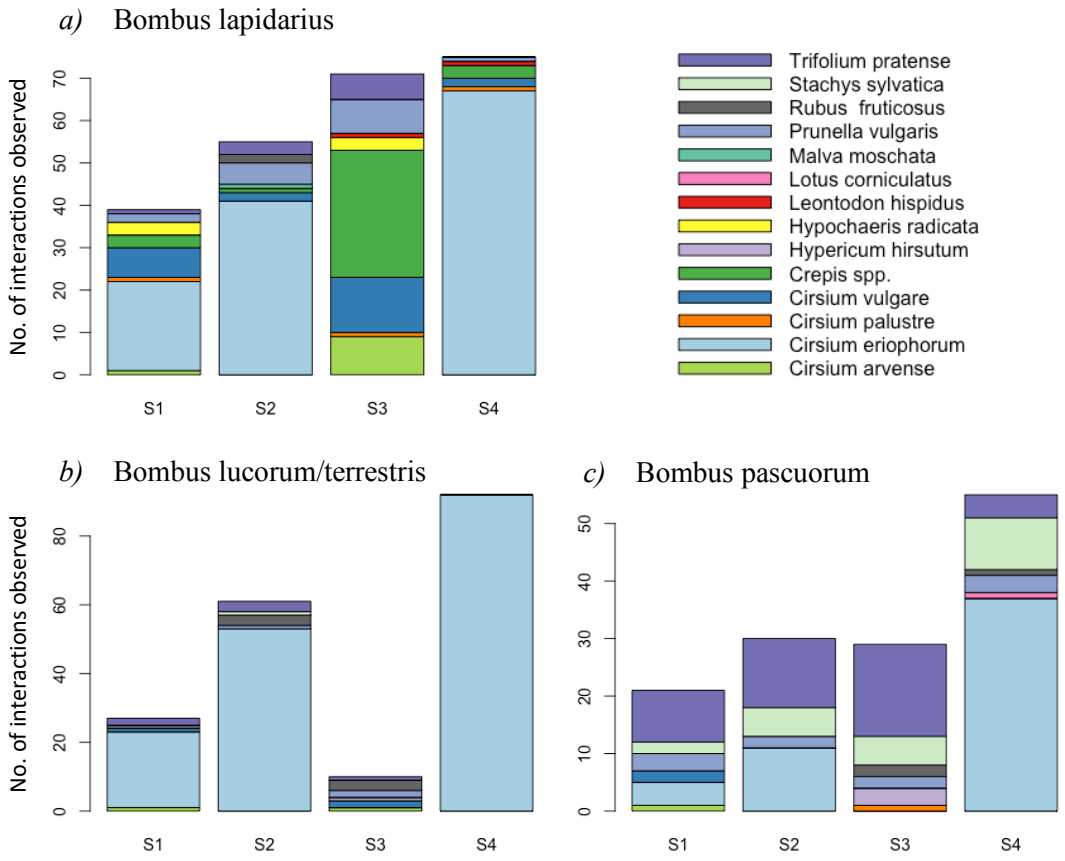


Figure 4.22 The interactions observed between plant species and the three key bumblebee species ***a)*** *B. lapidarius,* ***b)*** *B. lucorum/terrestris and* ***c)*** *B. pascuorum, in each survey. The three species have some overlap in the plant species that they visited. For each bumblebee species, there appears to be a similar set of plant species visited in S1, S2 and S4. In S3, it is visually clear that the three bumblebee species had different reactions to the loss of C. eriophorum.*

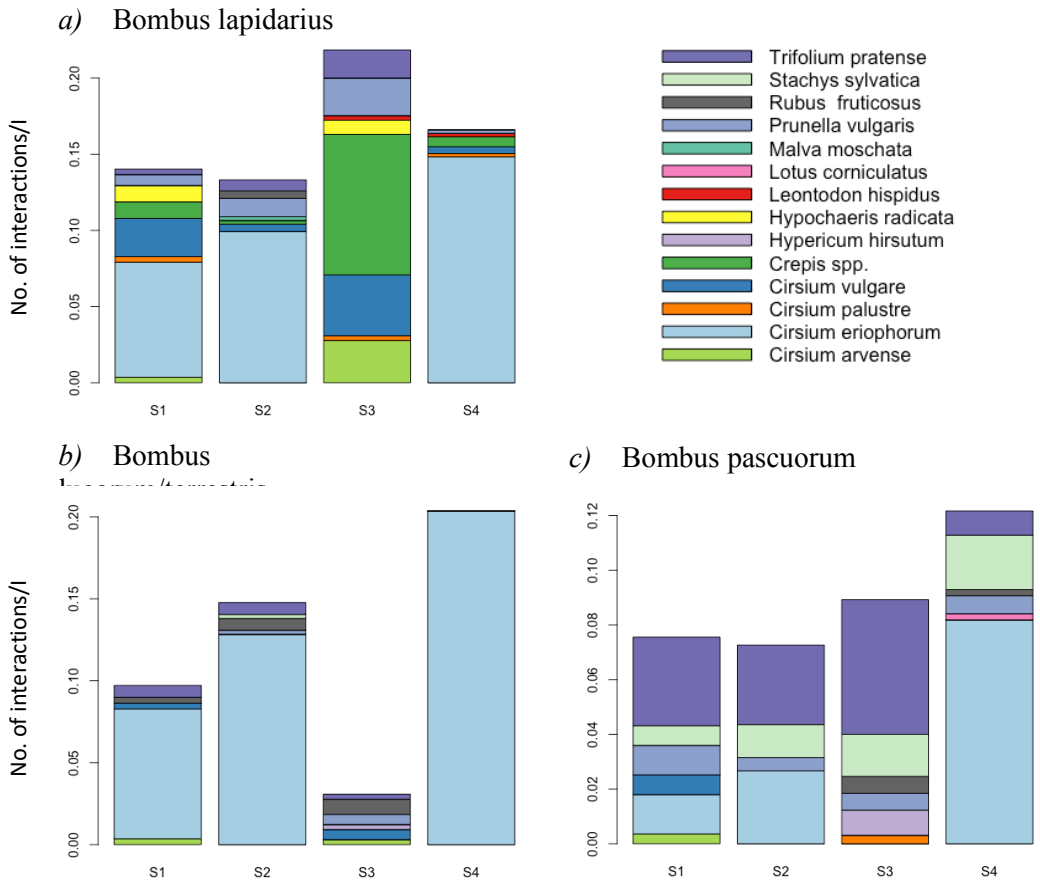


Figure 4.23 The interactions observed between plant species and the three key bumblebee species as in Figure 4.22 but here the number of interactions is normalised by the total number of interactions recorded in each survey. This demonstrates that the pattern of increasing numbers of interactions seen between S1, S2 and S4 in Figure 4.22 are not necessarily due to increasing numbers of bumblebee individuals as they are affected by the sampling effort during each survey.

We found that the proportions of interactions observed in S3 for *B. lapidarius* were significantly different to our expectation based on the H_1 Expansion and H_2 Replacement hypotheses (Table 4.9). In ecological terms, this suggests the *B. lapidarius* switched to feeding on other, new plant species, in new proportions when *C. eriophorum* was not available in S3. This implies that *B. lapidarius* is a more generalist species that is able to change its foraging patterns immediately following a change to the forage environment in order to make use of the plant species available. On the other hand, we found that the proportions of interactions observed in S3 for *B. lucorum/terrestris* and *B. pascuorum* were not significantly different to our expectation based on the H_1 Expansion hypothesis but were significantly different based on the H_2 Replacement hypothesis (Table 4.9). In ecological terms this means that these two bumblebee species continued to forage on the non-*C. eriophorum* plant species that they visited in S2 and did not significantly switch to feeding on other plant species. However, there appears to be a key difference between the responses

of *B. lucorum/terrestris* and *B. pascuorum*. Similar numbers of *B. pascuorum* were recorded in S2 and S3, in terms of counts and the proportion of total interactions (Figure 4.22 and Figure 4.23) suggesting that there was not a significant change in the number of *B. pascuorum* individuals foraging at the site. On the other hand, the number and proportion of interactions recorded for *B. lucorum/terrestris* was much lower in S3 compared to S2 (and also S1 and S4 - Figure 4.22 and Figure 4.23). This suggests that *B. lucorum/terrestris* left the site when *C. eriophorum* was removed, presumably to find foraging resources elsewhere. Only the small number that stayed continued to forage on the same non-*C. eriophorum* plant species as in S2.

Table 4.9 Results of the χ^2 and randomisation test for each bumblebee species under the alternative hypotheses; H_1 Expansion and H_2 Replacement. (= significant) B. lapidarius does not show predicted expansion, but also doesn't show predicted replacement. B. lucorum/terrestris and B. pascuorum do not show replacement, but do not differ from the expectation of expansion.*

	Species	χ^2 statistic	p-value
H_1	*B. lapidarius*	190.90 *	$1x10^{-4}$
	B. lucorum/terrestris	3.78	0.943
	B. pascuorum	0.41	0.463
H_2	*B. lapidarius*	714.56 *	$1x10^{-4}$
	B. lucorum/terrestris	36.57 *	$6x10^{-4}$
	B. pascuorum	3.70 *	0.015

4.5.d Indirect effects of the pulse perturbation: plants and pollinators

We found that, for pollinator species the weighted distance from *C. eriophorum* was a significant predictor of both the degree and number of interactions in F3. Degree in F2 (log_2 scaled) and the number of interactions in F2 (log_2 scaled) were significant covariates for absolute change in pollinator degree and absolute change in pollinator interactions respectively. These results indicate that there were knockon effects from the perturbation that impacted pollinators in terms of their degree in number of interactions. For plant species, only the degree in F2 (log_2 scaled) was a significant predictor of absolute change in degree and interactions; weighted distance was not a significant covariate. The results of the

GLMs are summarised in Table 4.10; relationships between the different variables are plotted in Figure 4.25 and Figure 4.24. Our models suggest that distance from *C. eriophorum* had a significant negative effect on absolute change in pollinator interactions and degree, backing our hypothesis that indirect effects are greatest in species that are closest to *C. eriophorum* in the network but only for pollinators. Plant species were not significantly impacted in this way.

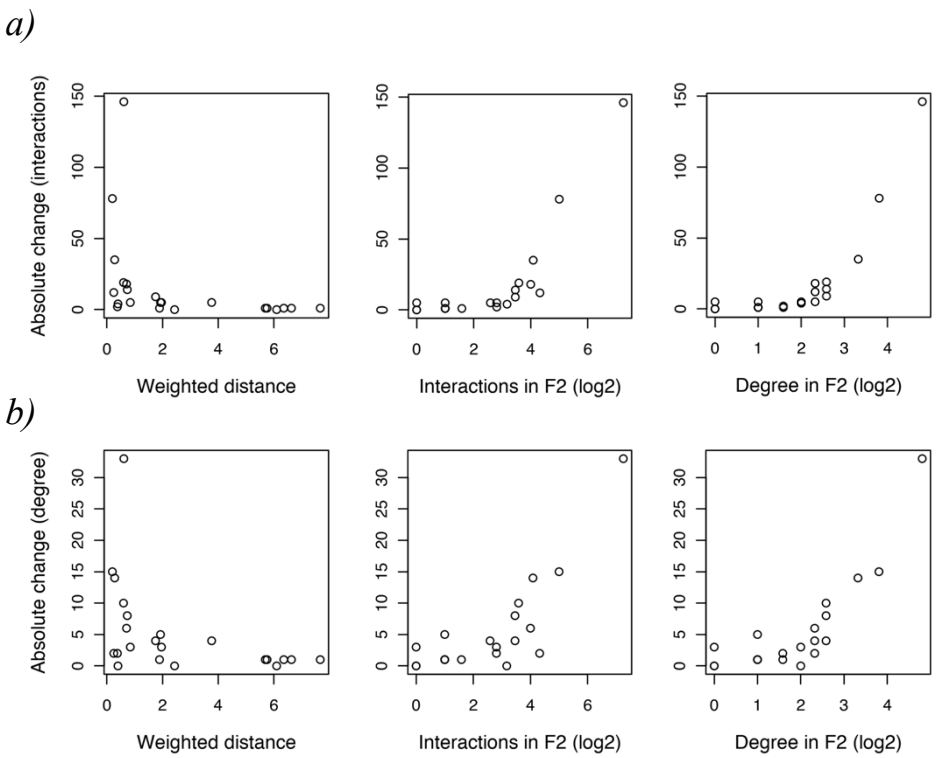
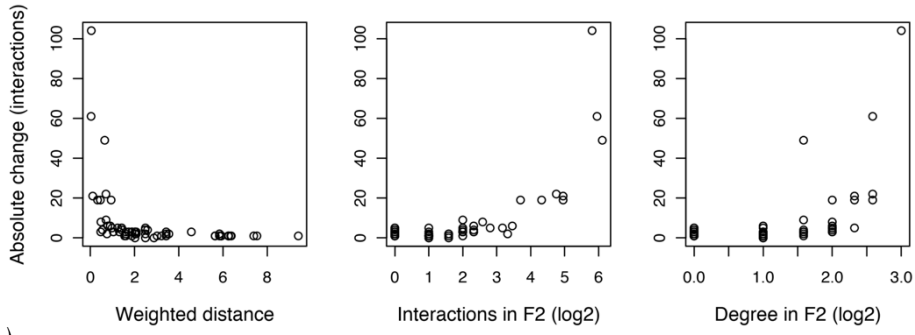


Figure 4.24 The relationships between the response variables: ***a)*** *absolute change in plant interactions and* ***b)*** *plant degree; and the explanatory variables: weighted distance from C. eriophorum over all four surveys (left column), total number of interactions (centre column – log2 scaled) and the observed plant degree (right column – log 2 scaled). C. eriophorum is not included in this data.*

a)



b)

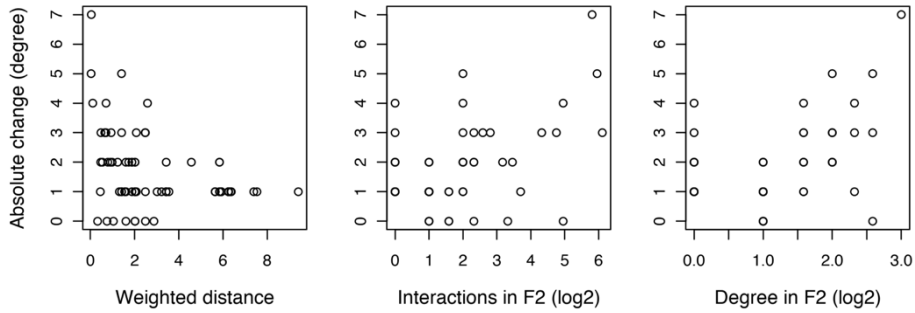


Figure 4.25 The relationships between the response variables: ***a)*** *absolute change in pollinator interactions and* ***b)*** *pollinator degree; and the explanatory variables: weighted distance from C. eriophorum over all four surveys (left column), total number of interactions (centre column – log2 scaled) and the observed pollinator degree (right column – log 2 scaled).*

Table 4.10 Summary of the results of the GLMs used to determine if weighted distance from C. eriophorum was a significant predictor of absolute change in degree or interactions of species as a result of the pulse perturbation. Significant results are highlighted in grey.

Dependent variable		**Independent variable estimate (*E*) and significance (*p*)**		
(Absolute change)		Weighted distance from C. eriophorum	log2(degree in F2)	log2(interactions in F2)
Plants	Degree	$E = -0.098$ $p = 0.199$	$E = 0.249$ $p = 2.0 \times 10^{-16}$	-
	Interactions	$E = -0.251$ $p = 0.031$	$E = 1.014$ $p = 3.9 \times 10^{-11}$	-
Pollinators	Degree	$E = -0.080$ $p = 0.094$	$E = 0.313$ $p = 0.009$	-
	Interactions	$E = -0.183$ $p = 8.2 \times 10^{-5}$	-	$E = 0.581$ $p = 2.0 \times 10^{-16}$

We found that for plant species, weighted distance from *C. eriophorum* was a significant predictor of both degree and interaction ratio (results summarised in Table 4.11). The greater the distance of a plant species from *C. eriophorum* the more likely it is for the degree and the number of interactions to decrease from F2 to F3 (interaction ratio decreases). On the other hand, weighted distance is not a significant predictor of interaction and degree ratio for pollinators. This is most likely because our data is strongly constrained by the sample size and for pollinators, there is great variation in degree ratio, particularly close to *C. eriophorum*.

Table 4.11 Summary of the results from the linear models using weighted distance to predict degree and interaction ratio. Weighted distance was found to be significant (*) for plant degree ratio and plant interactions ratio.

Dependent variable		**Independent variable: weighted distance**	
		Estimate (*E*)	Significance (*p*)
Plants	degree ratio	-0.127	0.009 *
	interaction ratio	-0.132	0.039 *
Pollinators	degree ratio	0.025	0.464
	interaction ratio	-0.012	0.795

4.6 Discussion

Studies of plant-pollinator networks can greatly contribute to our understanding of plant-pollinator communities as a whole and inform better conservation practices as a result. Only a small number of studies have carried out experimental manipulations on plant-pollinator communities. Most recently, one study looked at the effect of species removal from a plant-pollinator community in term of various network metrics (Brosi, Niezgoda and Briggs, 2017). Their study, compared control and manipulation sites each described by a single network. In our study, we carried out a manipulation (removal of *C. eriophorum*) at only one site, but described the network before and after the manipulation, and after a period of regrowth, in order to examine how the network changes over time and in response to the manipulation. We looked at network level effects in terms of standard network metrics, the

responses of bumblebee species and knock-on effects to other species. We expected that the network would significantly change as a result of the removal of the key plant species, *C. eriophorum*. We expected that this change would be detectable in various standard network metrics. We hypothesized that the loss of *C. eriophorum* would cause direct changes in the foraging patterns of the three most abundant bumblebee species associated with *C. eriophorum,* and that there would be detectable indirect effects on other species in the network.

We produced 4 networks; F1: 5 days before the manipulation; F2: the two days before the manipulation; F3: the three days after the manipulation; and F4, 8 days after the manipulation. We found that the F2 and F4 networks were more similar (positively correlated) that the F3 network, indicating a change in the network as a result of the perturbation, followed by healing of the network. However, we determined that the change in the numbers of *C. eriophorum* was the main driver of this as there was no significant difference in the correlation between W2 and W4 compared to W3 (the networks excluding *C. eriophorum*). We found that the patterns of the network metrics calculated for F1-F4 agreed with the significant changes in the corresponding network metrics found by Brosi, Niezgoda and Briggs, (2017) following the experimental removal of a pollinator species. In ecological terms, these results suggested an increase in competition between pollinator species due to a decrease in specialisation and niche differentiation.

To the best of our knowledge this is the first study to look at the temporal foraging effects of the removal of a key plant species on bumblebee species in the context of the plant-pollinator network. We find that the three most common bumblebee species at the site, *Bombus lapidarius, B. pascuorum* and the grouped species *B. lucorum/terrestris*, each exhibited distinct and different responses to the removal and regrowth of *C. eriophorum*. *B. lapidarius* and *B. pascuorum* both switched to feeding on other plant species at the site, though they did not switch onto the same plant species. There was also a slight reduction on the number of *B. pascuorum* suggesting that some of the individuals of this species might have gone elsewhere to forage. After regrowth, both species returned to feeding on *C. eriophorum* as well as a few other plant species, in similar ratios to before the manipulation. This suggests that for both of these bumblebee species, *C. eriophorum* was a preferred food source, but that in its absence, the bumblebees were able to quickly switch to feeding on other plant species at the site. The fact that species switched to different alternative food may be due to competition avoidance, or driven by biological constraints such a tongue length (Heinrich, 1976a; Inouye, 1980; Ranta and Lundberg, 1980). On the other hand, *B. lucorum/terrestris,* which was the most specialised on *C. eriophorum* almost completely

disappeared from the site after the removal of *C. eriophorum.* After regrowth, it returned in even greater numbers and fed exclusively on *C. eriophorum.* This suggests that for *B. lucorum/terrestris*, *C. eriophorum* was a preferred food source to the point that the individual bumblebees left the site when it was no longer available to forage elsewhere. Studies have shown that *B. terrestris* has a greater foraging range than other bumblebee species (Knight *et al.*, 2005; Kraus, Wolf and Moritz, 2009). They then returned when it was again abundant, perhaps because this foraging site was closer, or a greater nectar supply than whatever alternative they may have found during their absence from the site. *B. lucorum/terrestris* was the only bumblebee species that reacted in accordance with the knock-out extinction models in Chapter 2 that assume species will be 'knocked-out' of the network (Dunne, Williams and Martinez, 2002; Pocock, Evans and Memmott, 2012). The fact that these three, bumblebee species reacted so distinctly and differently to each other at the one site suggests that other pollinator species may also have their own foraging preferences that would result in differing responses to changes in their environment. The evidence here strongly suggests that treating pollinator species as the same when planning conservation outputs could not be the most effective way to ensure healthy populations of all the species in question.

We were able to model changes in interactions and species interacted with from before and after the perturbation for both plants and pollinators. Changes in plant degree, plant interactions and pollinator degree were significantly negatively affected by distance from *C. eriophorum* in the network. Species closer to *C. eriophorum* experienced greater changes in degree and interactions than species further away in the network. This suggests that indirect effects cascaded through the network to species not directly connected to *C. eriophorum.* This highlights the value of a network approach as focusing on only the species that are directly connected to *C. eriophorum* would miss any indirect effects.

One of the main limitations of this pulse perturbation study was that we were only able to reasonably carry out the experiment at one site. In order to compensate for this, we focused on the differences observed in a time series of networks, which also enabled us to ask questions concerning the often-overlooked issue of temporal changes in a plant-pollinator community. Despite our limited statistical power, we were able to show some clear effects of the removal of a key plant species. We therefore consider this study to have been a useful exploration of this type of experimental manipulation. We propose that future work should use replicated manipulations, perhaps considering the removal of other plant species also, at multiple sites as recently done by Biella *et al.* (2018).

Overall, we have shown that there are measurable changes in the structure of a plant-pollinator network following the removal of a key plant species. If the removed plant species is allowed to regrow, the network structure reverts to a state similar the pre-manipulation state. Following the removal of *C. eriophorum*, a major forage resource for several bumblebee species at this site, the three most abundant bumblebees showed distinct and different responses, ranging from full compensation by switching to alternative resources at the site, to leaving the site altogether, only to return when the preferred resource was again available. We believe that further studies should consider the potential for knock-on effects that cascade through the network as a result of species removal. Following in the footstep of Lopezaraiza-Mikel *et al.*, (2007) and Brosi, Niezgoda and Briggs, (2017), this study demonstrates the potential for experimental manipulations of plant pollinator communities to reveal previously undetected behaviours and to back up the burgeoning field of theoretical and observational studies of plant-pollinator networks.

Chapter 5

Flower constancy in foraging bumblebees

Abstract

Bumblebee individuals need to be able to forage efficiently and adapt to changes in floral resources over time if the colony is to survive. Therefore, at an individual and species level there should be a balance between foraging on the most rewarding plant species whilst retaining the ability to adapt when the foraging environment changes. Previous work has focused on understanding the mechanisms by which bumblebees improve the efficiency of their foraging and how they interact with floral cues. Recently, much of this work has been lab-based, with false or manipulated flowers and has focused on behavioural mechanisms in isolation, though in the past observational, field studies were common. In this chapter we examine the foraging patterns of bumblebee individuals in a resource abundant meadow. We were interested to see if wild foraging individuals showed 'flower constancy' as predicted by Darwin's Interference Hypothesis and optimal foraging theory. We were also interested to know if individuals switched between plant species whilst foraging as a potential behavioural mechanism for adapting to the floral resources available over time. We collected observations of foraging sequences (5 sequential flower visits) using a focal follow method, and quantified the available resources in the flight path of each individual bumblebee. Using this data, we developed foraging models to test the observed foraging behaviour for significant flower constant, switching and preference for floral cues. We found that:

a) the four most abundant bumblebee species observed foraged on the available plant species in significantly different proportions, but showed significant agreement in which plant species were most and least visited,
b) for all four bumblebee species, in both June and July, the proportion of individuals that were flower constant for 5 sequential flower visits was significantly greater than expected based on flower abundance,
c) we were able to quantify constancy (c) at the species level species and found that c did not significantly change over time,
d) there was evidence to suggest that bumblebee species switched between plant species based on a preference for floral cues.

5.1 Introduction

Bumblebees are reliant on flowering plant species for sustenance. Adult bumblebees visit flowering plants to collect pollen and nectar, which they feed on themselves, and also take back to the nest to feed larvae, the queen and other workers (Benton, 2006). Pollen provides protein and nectar provides sugar, water and amino acids (e.g. Carvalheiro *et al.*, 2014). Without these floral resources, individuals and the colony cannot survive. As demonstrated in Chapters 3 and 4, the floral resources available to bumblebees change throughout the flowering season. Therefore, it would appear not only advantageous, but essential that bumblebee colonies are able to respond to changing floral resources on timescales comparable with and potentially shorter than the lifetime of a worker bumblebee.

Bumblebees are estimated to have the one of the highest metabolic rates in the animal kingdom, 75% higher than that of hummingbirds (Goulson, 2010). Workers that use more energy foraging than the resources they consume and bring back to the colony put themselves and the colony at risk of starvation. The colony has some stores of food to support the queen and feed the larvae, but these are not sufficient to also support the foraging workers and must be replenished often (Goulson, 2010). Therefore, foraging bumblebees must feed often, and foraging behaviour must be profitable.

The floral resources available to foraging bumblebees can be highly variable and the distribution of rewards is unpredictable. Bumblebees are limited spatially by their foraging range from the nest; estimates are around 400m to 900m for common UK species (Knight *et al.*, 2005). When the nest site is chosen by the queen in the spring, the available resource will be different to what flowers later in the year. As the colony grows, different plant species will come into flower within the foraging range and will offer variable rewards based on age, time of day and environmental conditions (Pleasants, 1981; Herrera, 1990; Leiss and Klinkhamer, 2005; Cnaani, Thomson and Papaj, 2006). The bumblebee colony will outlive the flowering period of many plant species (as shown in Chapter 3) and therefore will have to forage on different plant species over time (See Figure 5.1).

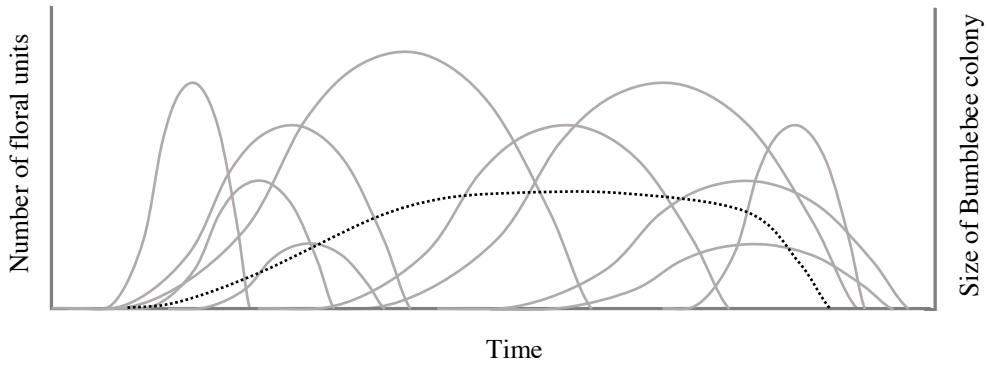


Figure 5.1 A schematic demonstrating the overlapping bumblebee colony lifespan (dotted line) and the number of floral units of nearby flowering plant species (solid grey lines). Note that the bumblebee colony lifespan overlaps with several flowering plant species, and that at any given point in time, the available floral resource (measured in floral units) is different. See Chapter 3 for detailed observational data showing overlapping flowering phenologies.

Optimal foraging theory suggests that foraging bumblebee individuals should preferentially forage on high reward plant species with low foraging costs (Dreisig, 1995). Foraging costs include flight distance and handling time *i.e.* the amount of time it takes for the bumblebee to access the nectar. In a similar vein, 'Darwin's Interference Hypothesis' states that insects preferentially visit a single species of plant as this enables them to work more quickly as they learn how to most efficiently access the nectar and pollen (summarised in Freeman, 1968). Therefore, it can be profitable to forage on one plant species, once it has been identified as profitable.

When a bumblebee individual forages on just one plant species, we refer to this behaviour as 'flower constancy' (also known as flower fidelity). Various studies have identified flower constancy in other insects such as honeybees, butterflies and hoverflies (see for example: Lewis, 1986; Goulson and Wright, 1998; Raine and Chittka, 2007). There is evidence of near absolute flower constancy in three alpine bumblebee species: *Bombus consobrinus, B. gerstaeckeri* and *B. brodmannicus*, perhaps unsurprisingly due to the short colony lifecycle associated with alpine species (Goulson, 2010).

In a variable foraging environment, there must be some balance between optimising foraging through flower constancy and switching to new plant species in order to adapt to changes in the floral resource over time. Bumblebees must be flexible, constant foragers. Such flexibility could occur at the colony level, where workers are allocated different foraging tasks. Some bumblebees could be constant on a known high reward plant while others could be 'scouts' which search for new profitable plants. To support this hypothesis, there is strong evidence for task allocation in social insects such as ants (Braendle et al.,

2003; Friedman and Gordon, 2016) and in stingless bees (Goulson, Derwent and Peat, 2005) where allocation is often based on individual body size (known as alloethism). It is possible that bumblebee worker size could be linked to forager task allocation (Shpigler *et al.*, 2013). Additionally, task allocation with constant foragers and scouts would require information to be shared at the colony level and adjustments made over time. Colony level communication in bumblebees is not well studied and is believed to be fairly simple (Dornhaus and Chittka, 2004) though complex communication mechanisms have been discovered in stingless bees (Nieh, 2004) and famously in honeybees (Frisch, 1950).

A second possibility is that flexibility occurs through the flower constancy of different aged workers. If a newly emerged forager learns to be flower constant on a currently-abundant plant species and remains constant throughout its lifetime, there could be natural turnover of flower constancy as new workers are born and older ones die. However, this could be risky if changes to the foraging environment are more rapid than worker turnover or if sudden changes occur.

In Chapter 4, bumblebee species reacted to the sudden loss of a main food source and particularly in the case of *Bombus lapidarius* it appeared that workers were able to switch to feeding on alternative plant species very quickly. This evidence suggests that individuals are able to adapt their foraging behaviour to feed on different plant species in the space of a couple of days. Therefore, a third possibility is that individual worker bumblebees switch between flower species whilst foraging and update their own foraging preferences based on their experience. A foraging bumblebee may be relatively flower constant on a known, high reward, plant species but will occasionally try other plant species to see if they are more rewarding. This possibility ties in with our current understanding of bumblebee learning. Studies show that bumblebees have accurate, long term memory and are able to transfer learned foraging skills to new flowers (Menzel and Erber, 1978; Laverty, 1994; Keasar *et al.*, 1996; Toda, Song and Nieh, 2009; Clarke *et al.*, 2013). In addition, there is evidence that individual bumblebees can identify and learn associations with floral cues (indicators of high reward). Studies have shown that naïve workers (those that have not yet foraged) have innate foraging preferences based on floral cues such as colour, scent, size and shape (symmetry) of flowers (Menzel and Erber, 1978; Lunau, Wacht and Chittka, 1996; Gumbert, 2000; Goulson, 2010) and that these can be honed or overwritten with foraging experience.

Bumblebee foraging behaviour has been well studied in both field and laboratory settings. Earlier studies into general aspects of bumblebee foraging behaviour, such as foraging distance, task allocation, exploitation of patchy resources and flight paths were

based on field observations (*E.g.* Free, 1955b; Manning, 1956; Laverty and Plowright, 1988; Thomson, 1996). A classic example of this type of work are a serious of observational studies by Bernd Heinrich in the 1970/80s focusing on bumblebees foraging in the wild (Heinrich 1976a, 1976b, 1979a, 1979b, 1983). Foraging behaviour of bumblebees was also described in detail by John Free (Free, 1955; Free, 1970). Many of the recent studies into bumblebee foraging behaviour have used captive bumblebee colonies in laboratory settings and have used false flowers to focus on the effect of floral cues including size (Goulson *et al.*, 1998), flower colour (Lunau, Wacht and Chittka, 1996; Heuschen, Gumbert and Lunau, 2005; Lunau, 2016) and ultra-violet markings (Papiorek *et al.*, 2016). It is also known that bumblebees are able to learn specific handling skills for complex flowers and recall these skills to foraging more efficiently when switching between flowers (Woodward and Laverty, 1992; Laverty, 1994, 1997). We wanted to look at foraging patterns of individual bumblebees in a natural setting where they have a choice of many different plant species to forage on.

Inspired by Heinrich's observational studies, we wanted to observe wild populations and follow individual bumblebees in order to better understand foraging patterns and how bumblebees might adapt to changes in their foraging environment. We were motivated to explore the foraging behaviour of bumblebees and how they might adapt to change because the foraging behaviour of individual insects is a fundamental component of the structure of plant-pollinator communities. As pointed out by Dupont, Trøjelsgaard and Olesen (2011), a network of interactions between plant and pollinator species is ultimately made up of networks of interactions between individuals through time. By understanding the foraging patterns and behaviour of individuals and species we can better inform our understanding and use of plant-pollinator network data.

In this chapter we present an analysis of bumblebee foraging patterns. We developed a novel protocol in which we followed bumblebee individuals for 5 consecutive flower visits; a foraging bout. We carried out our focal follows in a densely flowered meadow where the forage resource was diverse and mixed (not patchy). Our main aims were:

i. to determine if individuals exhibit flower constancy,
ii. to quantify flower constancy at the species level based on the tendency for bumblebee individuals to be flower constant
iii. to determine if there is evidence for task allocation based on alloethism
iv. to determine if bumblebee species showed a preference for floral cues when switching between plant species

We focused on 4 different floral cues; colour, symmetry, size and height. We chose the first three because of evidence in the literature that bumblebees can detect and show a preference for them. We additionally chose height because, having observed the foraging bumblebees at the site, we expected that this might be a possible driver. Based on our experience of 3 common bumblebee species in Chapter 4, we expected there to be significant differences in foraging patterns between different bumblebee species, and so we treat bumblebee species separately in our analyses and determine if there are significant differences. Having collected data for four abundant bumblebee species, in both June and July, we determined if constancy and foraging preferences were different across species and over time. Overall, this chapter is about focussing in on individual pollinators and understanding the dynamics of foraging behaviour because it is through the behaviour of individuals and species that plant-pollinator communities change over time.

5.2 Survey Methods

5.2.a The survey site: Avis meadow

The study site was Avis Meadow near Malmesbury in Wiltshire, UK (51.587169° N, -1.962163° W). Avis Meadow is one of two adjacent meadows that are part of the Ravensroost Wood Reserve, managed by the Wiltshire Wildlife Trust (Figure 5.2). Both meadows are classed as flower-rich grassland, though the larger, western meadow (approximately 5.5 hectares) in which we surveyed is particularly flower-rich. The site is surrounded by grassland meadows and arable farmland, with Ravensroost Wood 0.3km to the northwest, and Milbourne Common Wood 0.5km to the south. To the best of our knowledge, there were no comparably flower-rich sites within at least 1km and that therefore Avis Meadow provides the best resources for pollinators seeking a plentiful and diverse range of foraging resources. Apart from records of which species were present (collated by the Wiltshire Wildlife Trust) no extensive surveys had taken place on the site before.

Figure 5.2 Maps of the area indicating the location of Avis Meadow (A) where we surveyed and the adjacent wild flower meadow (B). In both the map view (left) and aerial view (right) Ravensroost Wood to the north and Sommerford Common Wood to the south can be seen. In the aerial view (right) the arable farmland that surrounds the site can be seen. Images: © OpenStreetMap contributors, openstreetmap.org accessed 20.10.18 and Imagery ©2018 Getmapping plc, Map data ©2018 Google, accessed 20.10.18.

5.2.b Focal follows of foraging bumblebees

In order to sample the foraging behaviour of bumblebees, a 'focal follow' method was devised in which a target bumblebee individual was followed for five flower visits (see Gursky, 2000; Quick, Rendell and Janik, 2008 for examples of other focal follow methods and Heinrich, 1976a, 1976b, 1979a for bumblebee observation methods). Three observers independently carried out focal follows: M. Bane, M. Pocock and R. James. Each observer would continuously walk through the meadow at a steady pace, searching for bumblebees. When a bumblebee was detected, it was followed for its next 5 flower visits. A visit was defined as landing on and attempting to feed from a flower. The species of each of the 5 flowers visited during the focal follow was recorded. The duration of the focal follow, from the first flower visit to the last, was recorded by the observer using a stopwatch. Once the bumblebee had visited the fifth flower, it was caught using a bumblebee net. From the net, it was transferred into a bumblebee holding device which consists of a clear plastic tube with netting over one end and a cushioned plunger that allows the bumblebee to be held still against the netting for close examination (Figure 5.3). The bumblebee species and caste were identified and the length (from the top of head to the tip of the tail) was measured using a standard ruler. The bumblebee was marked with a dot on each forewing using a non-toxic permanent marker pen before being released. Finally, the abundance of all plant species in the area 30cm either side of the flight path that the bumblebee had taken between the visited flowers was recorded; we call this the foraging patch (See Figure 5.4). Abundance estimates

were made by counting or extrapolating the number of floral units, using the same method as in Chapters 3 and 4 (floral units for species surveyed are summarised in Table 5.2). The patch data provides an indication of the flower species that were immediately available as foraging options to the bumblebee.

We surveyed for four days in June 2016 (6th-9th) and four days in July 2016 (18th-21st), in order to compare foraging patterns observed at the same site when the floral resource available was different. The focal follow method was tested on the first survey day (6th June 2016) with only 3 visits but it was quickly determined that 5 visits was easily achievable. From this point, the focal follow protocol (with 5 visits) was repeated continuously by 1 to 3 observers throughout the day from when the first bumblebees were spotted foraging (approximately 9:30am) until the number of foraging bumblebees began to decline at the end of the day (typically 3:30pm). Short rest breaks were taken but the aim was to maximise the number of focal follows carried out over the 4-day survey period in both June and July.

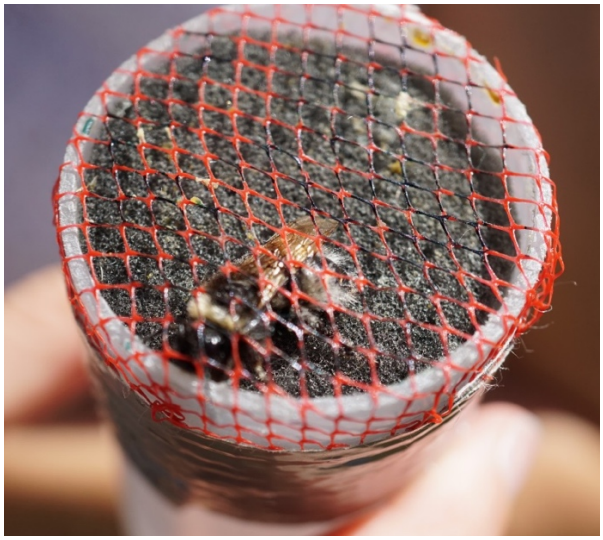


Figure 5.3 A bumblebee (Bombus hortorum worker) ready for examination and measuring in the bumblebee holding device

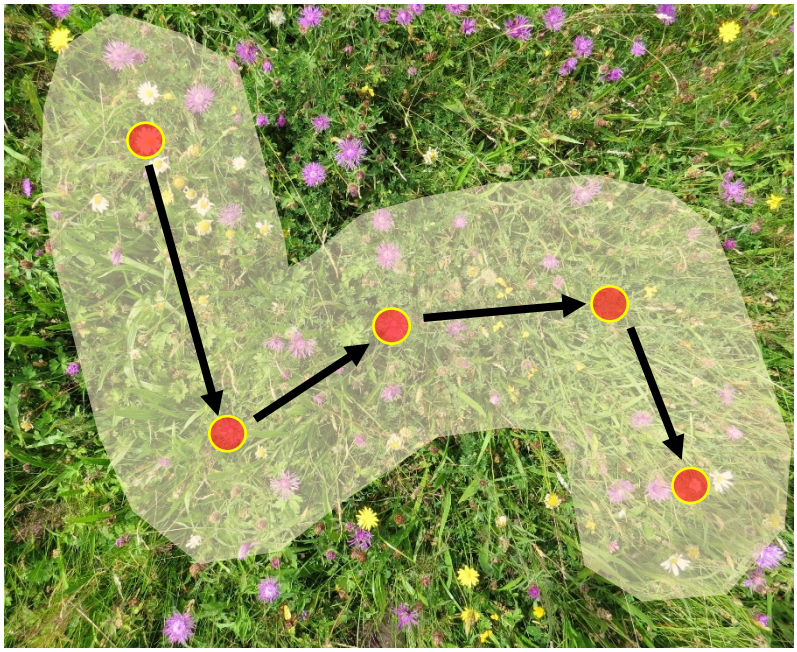


Figure 5.4 An aerial view of an imagined focal follow showing the flight path of the bumblebees, flower visits and the foraging patch surveyed. The red circles indicate the 5 flowers visited and the arrows show the path flown from one flower to the next. The pale area indicates the foraging patch (30cm either side of the flight path) within which the number of each plant species was recorded.

5.3 Overview of the data

A total of 261 (in June) and 188 (in July) focal follows were recorded. Each focal follow describes the 5 flowers visited by an individual bumblebee; a foraging sequence. For each focal follow, we know the duration of the observation, the flight distance of the bumblebee and the abundance of all plant species in the foraging patch. Four records were not included in the totals above: two corresponding to instances where the bumblebee was found to be marked when caught, indicating that it had already be surveyed and two records corresponding to male bumblebees (see section 5.4.a below). We assume that all remaining bumblebees were only surveyed once and so each focal follow corresponds to an individual bumblebee, as well as a single patch.

5.3.a Bumblebee species

We recorded five bumblebee species and the grouped species *Bombus lucorum/terrestris* (Table 5.1). *Bombus hypnorum* was only observed in June. All other species were observed in both June and July. Ten queen bumblebees were recorded (7 *B.*

terrestris, 1 *B. lapidarius* and 2 *B. pascuorum*), all in June. Only two males were recorded, both *B. pratorum*. Both were observed feeding on only 1 plant species, the first on *Ranunculus acris* in June and the second on *Centuria nigra* in July. There is evidence that males can have different foraging behaviour to queens and workers (Bertsch, 1984; Kraus, Wolf and Moritz, 2009). Therefore, records of males were excluded from our analysis. Our anlaysis will focus on the 4 most abundant species: *B. hortorum, B. lapidarius, B. lucorum/terrestris* and *B. pascuorum.*

Table 5.1 The number of focal follows of each of the 6 bumblebee species in June and July. As each focal follow corresponds to an individual bumblebee, the number of focal follows is also a measure of the abundance of each bumblebee species at the site.

Bumblebee Species	June	July
B. hortorum	27	29
B. hypnorum	8	0
B. lapidarius	43	51
B. luc/ter	102	15
B. pascuorum	58	92
B. pratorum	23	1

5.3.b Plant species

We recorded 19 plant species within the focal follow patches (See Table 5.2), 11 of which were visited by bumblebees. Only 8 plant species were present in both the June and July survey periods, and all plant species varied in abundance between the two survey periods (See Table 5.3). The floral resource available in each survey period was different. Therefore, we treat the June and July records separately.

*Table 5.2 Summary of the plant species recorded at the Avis Meadow site. Code refers to the unique species abbreviation used later in this chapter when listing focal follows. Floral unit (FU) describes our definition of a floral unit when counting species (see p 72 for visual key). Species that were present but never observed being visited by bumblebees are indicated by *.*

Species	Common name	Code	Floral Unit (FU)
*Heracleum sphondylium**	Hogweed	HW	head (capitulum)
Hypochaeris radicata	Common cat's-ear	CE	flower (capitulum)
*Leucanthemum vulgare**	Ox-eye daisy	OD	flower (capitulum)

Centaurea nigra	Black knapweed	KW	head (capitulum)
*Cerastium fontanum**	Common mouse-ear	ME	flower
*Stellaria graminea**	Lesser stitchwort	SW	flower
Lotus corniculatus	Bird's-foot trefoil	BT	cluster
Medicago lupulina	Black medick	BM	head
Trifolium pratense	Red clover	RC	head
*Vicia sativa**	Common vetch	CV	spike
Vicia cracca	Tufted vetch	TV	spike
Trifolium repens	White clover	WC	head
*Lathyrus pratensis**	Meadow vetchling	MV	flower
Ajuga reptans	Bugle	BG	spike
Prunella vulgaris	Self-heal	SH	spike
*Dactylorhiza fuchsia**	Common spotted orchid	SO	spike
Rhinanthus minor	Yellow rattle	YR	spike
Ranunculus acris	Meadow buttercup	BC	flower
*Potentilla erecta**	Tormentil	TT	flower

Table 5.3 The abundance of the 19 plant species surveyed within the focal follow patches within Avis Meadow. Total floral units (FU) gives the number of floral units counted within all foraging patches combined. No. of patches refers to how many patches a given plant species was recorded in. FU/m is equal to the number of floral units (FU) per metre of the total patch length in each month (335m in June and 306m in July).

	June			**July**		
Plant Species	Total floral units (FU)	No. of patches	FU/m	Total floral units (FU)	No. of patches	FU/m
R. minor	11376	254	31.66	135	30	0.43
L. corniculatus	4486	188	13.89	2469	159	8.02
R. acris	4427	244	12.77	176	67	0.53
M. lupulina	1493	63	3.89	215	20	0.70
T. pratense	1074	175	3.16	2218	163	6.89
H. radicata	242	91	0.69	243	80	0.76
L. vulgare	113	60	0.30	338	79	1.04

C. fontanum	113	29	0.32	11	3	0.03
A. reptans	49	17	0.18	0	-	-
S. gramienea	7	2	0.02	0	-	-
D. fushii	6	5	0.02	0	-	-
V. sativa	2	1	<0.01	0	-	-
C. nigra	0	-	-	3698	176	11.50
V. cracca	0	-	-	533	67	1.54
P. vulgaris	0	-	-	264	68	0.79
T. repens	0	-	-	86	26	0.23
P. erecta	0	-	-	79	11	0.20
H. spondylium	0	-	-	12	3	0.03
L. pratensis	0	-	-	14	8	0.05

5.4 Analysis Methods

5.4.a Differences in the foraging preferences of four bumblebee species

Here we focus on the four species for which we have most data: *Bombus hortorum, B. lapidarius, B. lucorum/terrestris* and *B. pratorum.* The last three in this list are the same species focused on in Chapter 4, which had different foraging preferences at the Bushey Norwood site (Chapter 4). Therefore, we expected to see a difference in foraging preferences across the four bumblebee species observed at Avis Meadow in this chapter. We used two different methods to measure the similarity of foraging preferences between bumblebee species. First, we determined if the proportion of visits to different plant species was significantly different across bumblebee species. We tested this using a chi-squared (χ^2) test with simulated p-values (to account for expected counts < 5), available in the 'chisq.test' function in R (R Core Team, 2017). We used only the first visit recorded for each bumblebee individual (summarised in Table 5.4 and Table 5.5) to avoid issues of independence and pseudo replication within a sequence (Hurlbert, 1984). We analysed June and July data separately.

Table 5.4 The number of visits to each plant species from each of the 4 most abundant bumblebee species (based on the first visit in each floral sequence recorded only) in June.

June	*B. hortorum*	*B. lapidarius*	*B. luc/ter*	*B. pascuorum*
R. acris	0	0	4	0
A. reptans	0	0	0	2
M. lupulina	0	0	0	1
L. corniculatus	0	25	30	4
H. radicata	0	5	1	0
T. pratense	1	0	1	6
R. minor	26	13	66	45

Table 5.5 The number of visits to each plant species from each of the 4 most abundant bumblebee species (based on the first visit in each floral sequence recorded only) in July.

July	*B. hortorum*	*B. lapidarius*	*B. luc/ter*	*B. pascuorum*
L. corniculatus	0	6	1	16
H. radicata	0	0	1	0
C. nigra	1	37	11	13
T. pratense	21	7	1	43
P. vulgaris	1	0	0	5
V. cracca	2	0	0	12
T. repens	0	1	0	1
R. minor	4	0	1	2

We also wanted to know if bumblebee species showed similar preferences for plant species. For example, was the most visited plant species the same for all bumblebees? To test this we first normalised the proportion of visits to each plant species by the number of interactions observed for each bumblebee species and then calculated the intra-class correlation coefficient (ICC) for 'agreement' from the package 'irr' (Gamer and Lemon, 2012) in R (R Core Team, 2017). The ICC is typically used to measure the 'agreement' and 'consistency' between rankings or scores given by different raters of judges (Bartko, 1966). Both ICC measures lie between 0 and 1 where 0 is no agreement/consistency and 1 is complete agreement/consistency. In our case, the four bumblebee species were raters, the

proportion of individuals observed feeding on each plant species were the scores and we tested to see if bumblebees significantly agreed in terms of the proportion of individuals that fed on each plant species.

5.4.b Do bumblebees tend to switch or stay flower constant?

If a bumblebee was recorded visiting only one plant species for the 5 observed visits it was flower constant: a 'non-switcher'. Conversely, if a bumblebee individual visited more than one flower species during a focal follow it was a 'switcher'. We assigned each bumblebee individual as a switcher or non-switcher and used a chi-squared test to determine if the ratio of switchers to non-switchers was significantly different across the 4 most abundant bumblebee species. We also used a chi-squared test to determine if the proportion of switchers to non-switchers was significantly different between June and July for each species (with simulated p-values where necessary).

5.4.c A floral abundance weighted foraging model

So far we have examined bumblebee foraging, ignoring the foraging environment; what plant species were available for each individual bumblebee to feed on. Therefore, we developed a plant-abundance foraging model, using the patch data, in order to test whether the observed ratio of switcher to non-switcher bumblebees was significantly different from our expectation taking the foraging environment into account. The model works as follows: for each observed patch we generated 1,000 foraging sequences by randomly selecting 5 plant species from within the patch to generate each sequence (only selecting plant species we know were visited by bumblebees at the site). In the first instance, we weighted the probability of selecting a plant species by the proportion of floral units for each species in the given patch. In the second instance, we weighted the probability of selecting a plant species by its proportional nectar provision. We used nectar measurements from a recent study (Baude *et al.*, 2016), summarised in Table 5.6. We call our two models the 'floral units random foraging model' and the 'nectar provision random foraging model'. Having generated 1,000 null foraging sequences for each observed patch we determined the expected probability of a bumblebee foraging in each patch being a 'non-switcher' (N) or 'switcher' (S) based on the mean ratio of N to S null sequences generated. We used a Chi-squared test to determine if the observed N:S ratio was significantly different from the expected N:S ratio for floral unit abundance driven foraging and nectar provision foraging.

5.4.d Quantifying bumblebee constancy

We next sought to quantify non-switching; to calculate a value of 'constancy' that describes the tendency of each bumblebee species to remain constant on one plant species. To do this, we developed a second model that we call the 'constancy weighted foraging model'. This model takes the first plant species observed in each foraging sequence and generates a sequence of 4 subsequent plant species based on the patch specific abundance of plants (measured in floral units or nectar) and a non-switching constant (c). The non-switching constant c increases the probability of the next plant in the sequence being the same as the plant just visited. The model works as follows: we picked a patch at random from the observed data and preserved the first flower visited. Subsequent flowers in the sequence were picked with probability weighted by the abundance of each species in the chosen patch while the switching constant (c) increased the probability of the next plant in the sequence being the same as the previous by inflating the abundance ($0 \leq c \leq 1$). For example, if $c = 0.4$, and the previous plant was YR, the next plant was selected based on the patch abundances with an extra 40% for YR (all abundances are re-normalised) as shown in Figure 5.5.

Table 5.6 Mean nectar content per floral unit for each plant species at Avis Meadow. Our definitions of floral units differ in some cases to the definitions used by Baude et al. (2016) and so were converted (*). All of the 19 plant species identified within Avis Meadow were represented in this study, except for Dactylorhiza fuchsia (an orchid that does not contain nectar).

	Mean nectar sugar content in µg/floral unit	
Species	Baude *et al.* (2016)	Avis Meadow 2017
Rhinanthus minor	108.90	235.22*
Lotus corniculatus	61.82	137.24*
Ranunculus acris	78.83	78.83
Medicago lupulina	1.63	1.63
Trifolium pratense	116.86	116.86
Hypochaeris radicata	14.46	14.46
Leucanthemum vulgare	15.81	15.81
Cerastium fontanum	26.93	26.93
Ajuga reptans	155.06	1550.60*
Stellaria graminea	17.05	17.05
Vicia sativa	300.34	300.34
Centaurea nigra	198.99	198.99
Vicia cracca	484.40	3167.98*
Prunella vulgaris	138.62	780.43*

Trifolium repens	48.97	48.97
Potentilla erecta	33.28	33.28
Heracleum sphondylium	98.17	98.17
Lathyrus pratensis	952.69	952.69
Dactylorhiza fuchsii	na	na

In the first instance the number of floral units was used as the measure of abundance in the second, nectar provision. This model was used to produce 25,000 null foraging sequences for each bumblebee species in June and July (approximately 100 per patch). Each null foraging sequence was classified as switcher or non-switcher and the total number of visits generated for each plant species was recorded. This was repeated with different values of *c* to find the one that generated a ratio of switcher to non-switcher sequences most similar to the observed data in both June and July separately; the value of *c* that best describes the observed constacy. We used a Wilcoxon signed rank test to determine if there was a significant difference between the *c* values calculated using floral units and those calculated using nectar provision. We also used a Wilcoxon signed rank test to determine if there was a significant difference between the *c* values in June and July for each species.

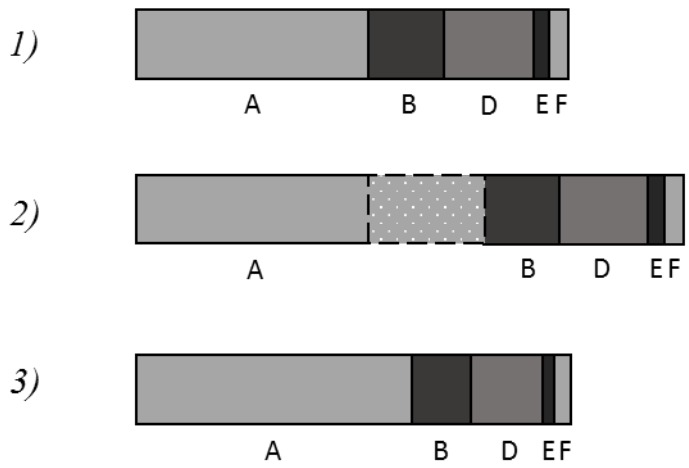


Figure 5.5 A schematic demonstrating how the non-switching constant (c) inflates the probability of the next plant in a sequence being the same as the previous. Step ***1)*** *shows the proportional abundances of flowers A, B, D, E and F in a given patch. Say the first visit in the sequence was to plant A and the non-switching constant is 0.5. In step* ***2)*** *the abundance of A is inflated by an extra 50% (c=0.5). In step* ***3)****, the abundance of all plant species are re-normalised, taking the inflated A into account. The next plant in the sequence can now be calculated from the relative abundances in step* ***3****, making A more likely to be picked than before. This method is applied to pick each subsequent plant species, each time starting with the plant abundances as observed in* ***1.***

5.4.e Evidence for alloethism: size related task allocation

One avenue we were interested in exploring was whether there was a difference in body size between bumblebees that were observed switching, and those that were observed being constant. We hypothesized that large workers were more likely to be constant, based on the assumption that larger workers are more likely to be allocated foraging tasks and therefore have greater foraging experience and know which plant species are the most rewarding. We used an unpaired t-test to test our hypothesis for each species.

5.4.f Evidence for floral cue preference in switching

The final aim of our analysis was to determine if bumblebee individuals that switched between plant species showed a preference for floral cues. For this analysis, we examined only the floral sequences of bumblebees that were observed switching between plant species (Table 5.7). First, we categorised each plant species in Avis Meadow according to 4 different floral cues; colour, symmetry, size and height. These are summarised in Table 5.8. We then determined which bumblebee individuals showed cue preference (*cp*) for each of the four cues based on the observed foraging sequences. For example, take the foraging sequence: YR, YR, BT, YR, BT where YR is yellow rattle (*Rhinanthus minor*) and BT is birds foot trefoil (*Lotus corniculatus*). In terms of the cue 'colour', the sequence translates as: Yellow, Yellow, Yellow, Yellow, Yellow. In this example, the bumblebee switched between plant species but all 5 visits were to yellow plants, therefore we would class this individual as having a cue-preference for colour (cp_{colour}). We summarised cue-preference in species (*CP*) as the fraction of individuals classed as having cue preference, for each of the 4 cues, in June and July.

We then used a modified version of the floral units random foraging model (see section 5.4.c) to test if the observed species cue-preference (CP_{obs}) was significantly higher than expected based on the foraging environment (using the patch data). In this version of the model, for each patch we generated a foraging sequence with the probability of picking each plant weighted by its proportional patch abundance (as before). We determined the fraction of these null sequences that showed cue preference (for each of the 4 cues) to get the null cue-preference (CP_{null}) for each species. We repeated the model 9999 times resulting in a distribution of CP_{null} values for each species in June and July. From these distributions, we determined if CP_{obs} was significant.

Table 5.7 The number of bumblebee individuals that switched in their observed floral sequence. Numbers of switchers for B. hortorum in July and B. lucorum/terrestris in June were too small for our switching analysis so are not examined further.

	Number of individuals observed switching	
	June	**July**
B. hortorum	1*	12
B. lapidarius	14	14
B. lucorum/terrestris	30	2*
B. pascuorum	15	54

Table 5.8 Summary of categorised floral cues for the species visited by bumblebees at Avis Meadow. Each floral cue has three categories associated with it.

Plant speices	**Colour**	**Symmetry**	**Size**	**Height**
Rhinanthus minor	Yellow	Bilateral	Small	Short
Lotus corniculatus	Yellow	Bilateral	Small	Short
Ranunculus acris	Yellow	Radial	Medium	Tall
Medicago lupulina	Yellow	Radial	Small	Short
Trifolium pratense	Purple	Radial	Medium	Medium
Hypochaeris radicata	Yellow	Radial	Medium	Tall
Ajuga reptans	Purple	Bilateral	Small	Short
Centaurea nigra	Purple	Radial	Large	Tall
Vicia cracca	Purple	Bilateral	Small	Medium
Prunella vulgaris	Purple	Bilateral	Small	Short
Trifolium repens	White	Radial	Medium	Medium
Lathyrus pratensis	Yellow	Bilateral	Small	Medium

5.5 Results

5.5.a Differences in the foraging preferences of four bumblebee species

We found a that there was a significant difference in the ratio of plant species visited by the 4 most abundant bumblebee species (first visit only) in both June: $\chi^2 = 88.34, \mathrm{p} = 1 \times 10^{-4}$, and in July: $\chi^2 = 112.50, \mathrm{p} = 1 \times 10^{-4}$ (see Figure 5.6). This confirms our expectation that the bumblebee species were not visiting the same plant species in similar proportions and suggests that they had different foraging preferences, as we also saw in Chapter 4.

We found there was significant intra-class agreement in the proportion of individuals feeding on different plant species in June: $ICC = 0.716, p = 8.2 \times 10^{-5}$. Conversely, intra-class agreement was significantly low in July: $ICC = 0.350, p = 0.028$. This tells us that although bumblebee species were visiting plant species in significantly different proportions (see above), there was significant agreement in which plant species received a high proportion of visits and which received a low proportion of visits, but only in June. This can been seen in Figure 5.6.

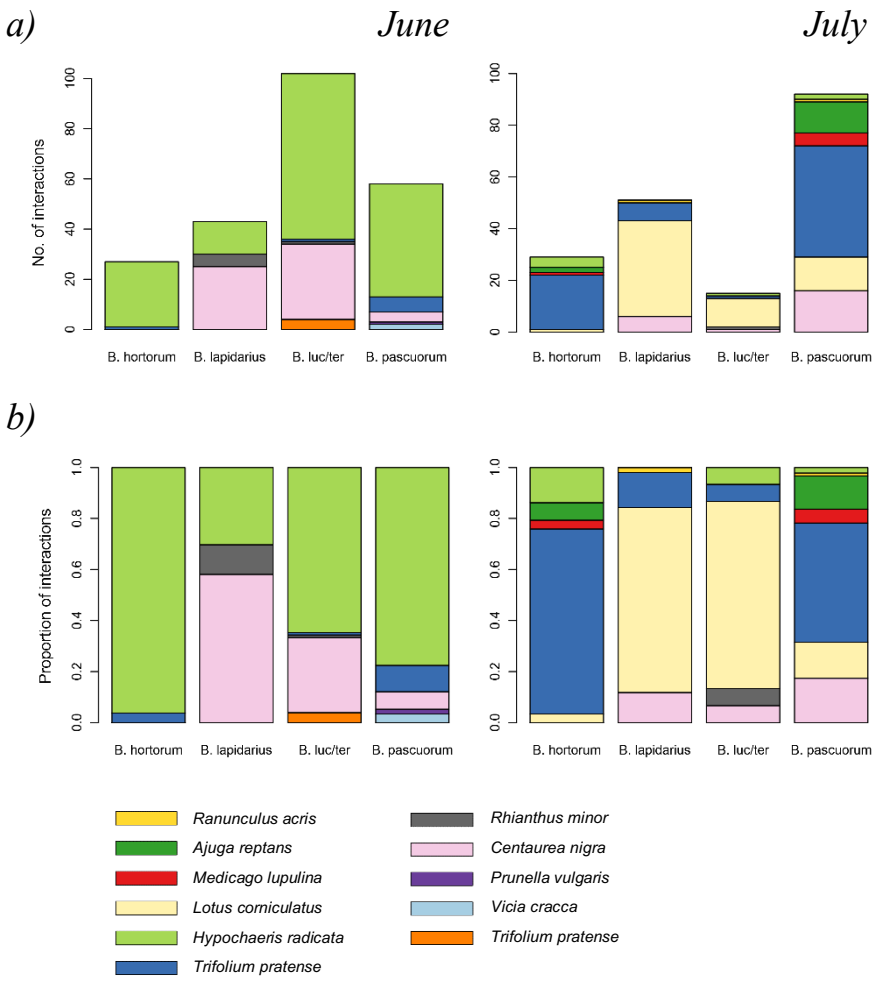


Figure 5.6 ***a)*** *The number of interactions and* ***b)*** *the proportion of interactions recorded between the four bumblebee species and the plant species at the Avis Meadow site in June (left hand column) and July (right hand column) based on the first recorded visit for each bumblebee individual only.*

5.5.b Do bumblebees tend to switch or stay flower constant?

Out of the 261 bumblebees recorded in July, 200 were non-switchers (77%) and 61 were switchers (23%). In July 188 bumblebees were recorded, 106 of which were non-switchers (56%) and 82 were switchers (44%). When broken down by species, the proportion of non-switchers was greater than non-switchers in all cases except for *B. pascuorum* in July where 59% were switchers (Figure 5.7). We found that the ratio of non-switchers to switchers (N:S) was significantly different across the four bumblebee species in both June: $\chi^2 = 8.54, \mathrm{df} = 3, p = 0.036$, and in July: $\chi^2 = 19.55, p = 2.1 \times 10^{-4}$. This suggests that the four species have different levels of constancy, whether that is controlled at a colony or individual level.

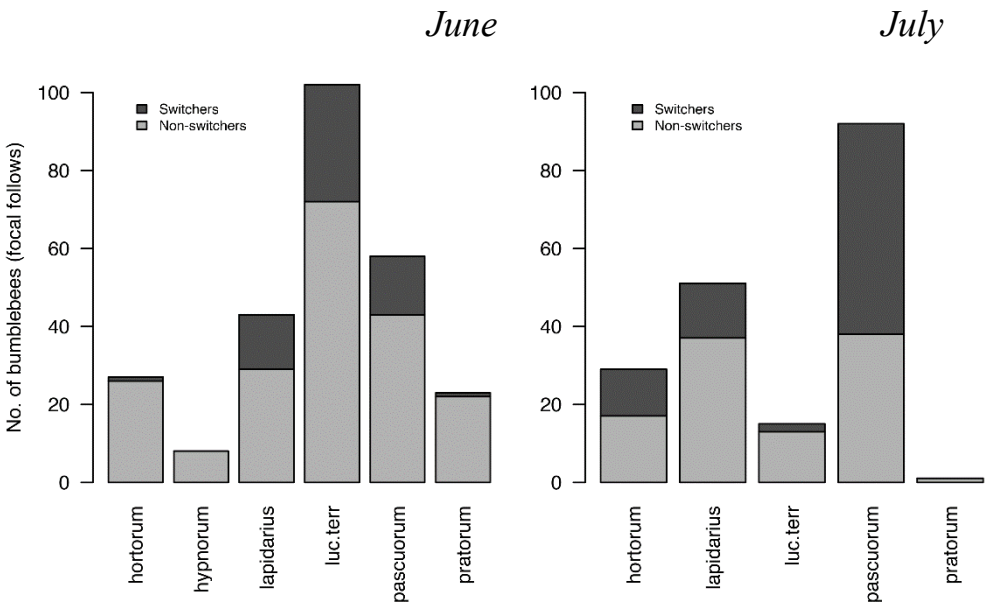


Figure 5.7 The number non-switcher bumblebees (light grey) observed during focal follows was greater than the number of switchers (dark grey) in both June (left hand plot) and July (right hand plot), though the difference was less pronounced in July and not true for Bombus pascuorum.

We found that there was no significant difference between the June and July ratio of non-switchers to switchers (N:S) for *B. lapidarius* ($\chi^2 = 0.10\ \mathrm{df} = 1, p = 0.754$) and *B. lucorum/terrestris* ($\chi^2 = 1.70,\ p = 0.235$). On the other hand, there was a significant difference between the June and July N:S ratio for *B. hortorum* ($\chi^2 = 9.12, \mathrm{df} = 1,\ p = 0.003$) and *B. pascuorum* ($\chi^2 = 14.15, \mathrm{df} = 1, p = 1.6 \times 10^{-4}$). This suggests that the foraging environment (what plant species are available), which changes over time, plays a role in the foraging behaviour of some bumblebee species and bumblebees may adapt to the foraging environment by adopting different levels of constancy.

5.5.c A floral abundance weighted foraging model

From the results of our foraging sequence simulation model we determined that the observed non-switching and switching ratios did not match the available floral resource and were therefore not a result of random foraging. The observed ratio of non-switcher to switcher bumblebee individuals was significantly different from the expected ratio produced by our floral units weighted model and nectar provision model for all four bumblebee species in June (Table 5.9) and July (Table 5.10).

Table 5.9 Summary statistics from the floral units random foraging model. In all cases, χ^2 is significant: $p < 0.001$ (*). Therefore the observed ratio of non-switchers to switcher (N:S) is significantly different from the expected ratio based on floral units or nectar provision weighted foraging.

Floral Units		*B. hortorum*	*B. lapidarius*	*B. luc/ter*	*B. pascuorum*
June	Observed N:S	26 : 1	29 : 14	72 : 30	43 : 15
	Expected N:S	0.14 : 0.86	0.08 : 0.92	0.10 : 0.90	0.10 : 0.90
	χ statistic	150.28*	217.4*	421.72*	257.24*
July	Observed N:S	17 : 12	37 : 14	13 : 2	38 : 54
	Expected N:S	0.09 : 0.91	0.06 : 0.94	0.09 : 0.91	0.05 : 0.95
	χ statistic *	90.49*	413.87*	112.34*	253.79*

Table 5.10 Summary statistics from the nectar provision random foraging model. In all cases, χ^2 is significant: $p < 0.001$ (*). Therefore the observed ratio of non-switchers to switcher (N:S) is significantly different from the expected ratio based on floral units or nectar provision weighted foraging.

Nectar Provision		*B. hortorum*	*B. lapidarius*	*B. luc/ter*	*B. pascuorum*
June	Observed N:S	26 : 1	29 : 14	72 : 30	43 : 15
	Expected N:S	0.39 : 0.61	0.19 : 0.81	0.27 : 0.73	0.29 : 0.71
	χ^2 statistic	36.96*	67.13*	95.06*	59.10*
July	Observed N:S	17 : 12	37 : 14	13 : 2	38 : 54
	Expected N:S	0.20 : 0.78	0.14 : 0.86	0.18 : 0.82	0.17 : 0.83
	χ^2 statistic	26.73*	148.72*	48.43*	36.95*

In all cases, the observed proportion of non-switchers was much greater than the expected proportion of non-switchers based on the foraging environment. This tells us that non-switching behaviour was not driven by highly abundant plant species or nectar sources and the observed proportion of non-switchers was much higher than we would expect if foraging was random within each patch.

5.5.d Quantifying bumblebee constancy

We found that the non-switching constants (c) that best describe the foraging behaviour of each bumblebee species lie in the range $0.79 \leq c \leq 0.97$ in June and $0.65 \leq c \leq 0.93$ in July (results for each bumblebee species summarised in Table 5.11). A

Wilcoxon signed-rank test indicated that there was no significant difference between c calculated using floral units and c calculated using nectar ($W = 34, p = 0.875$) and no significant difference between c for each bumblebee species in June and July ($W = 41, p = 0.371$). This indicates that each bumblebee species is approximately equally constant in June and July despite changes in the floral resource and that constancy can be calculated using floral units or nectar provision for the same result.

Table 5.11 The non-switching constant (c) calculated for each bumblebee species in June and July. The constancy weighted foraging model was used to calculate 'c' with both floral units and nectar provision as a measure of the abundance of plant species available for foraging on.

	Non-switching constant in June		**Non-switching constant in July**	
	Floral Units	Nectar	Floral Units	Nectar
B. hortorum	0.97	0.96	0.79	0.80
B. lapidarius	0.83	0.82	0.85	0.87
B. luc/ter	0.81	0.78	0.82	0.93
B. pascuorum	0.86	0.80	0.65	0.65

5.4.e Evidence for alloethism: size related task allocation

When comparing the size (body length) of non-switchers and switchers, we found that *B. hortorum, B. lapidarius and B. lucorum/terrestris* non-switchers were not significantly larger than switchers. However, *B. pascuorum* non-switchers were significantly larger than switchers in June ($\mathrm{t} = 2.35, \mathrm{df} = 47.79, p = 0.01$) and July ($\mathrm{t} = 2.078, \mathrm{df} = 75.56,\ p = 0.02$). Our hypothesis that larger bumblebees are more likely to be flower constant based on greater foraging experience stands true for one out of the 4 bumblebee species observed.

5.4.f Evidence for floral cue preference in switching

We found evidence to suggest there was cue preference, for at least 1 cue, in each of the four bumblebee species (summarised in Table 5.12). *Bombus hortorum* showed significant cue preference for colour in July (there was not enough data for June). Both *Bombus lapidarius* and *Bombus lucorum/terrestris* showed significant preference for all four cues in June. However, in July there was not significant preference for any of the cues for

Bombus lapidarus (not enough data for *Bombus lucorum/terrestris*). *Bombus pascuorum* showed significant preference for size and height in June, and colour and height in July. See Tables 5.13 to 5.16 for summary statistics and Figure 5.8 for a visual summary of these results, showing the distributions CP_{null} values compared to CP_{obs}.

Table 5.12 Summary of the floral cues for which there is evidence of significant constancy, for each of the four bumblebee species, in June and July. NA indicates that there was not sufficient data to test. None indicates that no cues were significant.

	June	**July**
B. hortorum	NA	colour
B. lapidarius	colour / symmetry / size / height	none
B. lucorum/terrestris	colour / symmetry / size / height	NA
B. pascuorum	symmetry / size / height	colour / height

Table 5.13 Summary statistics from the floral units random foraging model for Bombus hortorum individuals that switched between plant species. In this and the following 3 tables (5.14 to 5.16): significant values (*) indicate that cue preference (CP_{obs}) was significantly higher than expected based on random foraging, taking the foraging environment into account.

B. hortorum		**Colour**	**Symmetry**	**Size**	**Height**
July	CP_{obs}	0.75*	0.25	0.00	0.08
	Significance *(p)*	5×10^{-5}	0.836	1.000	0.776

Table 5.14 Summary statistics from the floral units random foraging model for Bombus lapidarius individuals that switched between plant species.

B. lapidarius		**Colour**	**Symmetry**	**Size**	**Height**
June	CP_{obs}	0.93*	0.93*	0.93*	0.93*
	Significance *(p)*	0.045	1×10^{-4}	1×10^{-4}	1×10^{-4}
July	CP_{obs}	0.96*	0.73*	0.73*	0.73*
	Significance *(p)*	1×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-4}

Table 5.15 Summary statistics from the floral units random foraging model for Bombus lucorum/terrestris individuals that switched between plant species.

B. lucorum/terrestris		**Colour**	**Symmetry**	**Size**	**Height**
June	CP_{obs}	0.96*	0.73*	0.73*	0.73*
	Significance *(p)*	4×10^{-4}	1×10^{-4}	1×10^{-4}	1×10^{-4}

Table 5.16 Summary statistics from the floral units random foraging model for Bombus pascuorum individuals that switched between plant species.

B. pascuorum		**Colour**	**Symmetry**	**Size**	**Height**
June	CP_{obs}	0.27	0.40*	0.47*	0.47*
	Significance *(p)*	0.999	0.049	0.020	0.014
July	CP_{obs}	0.52*	0.20	0.02	0.15*
	Significance *(p)*	1×10^{-4}	0.690	0.911	0.043

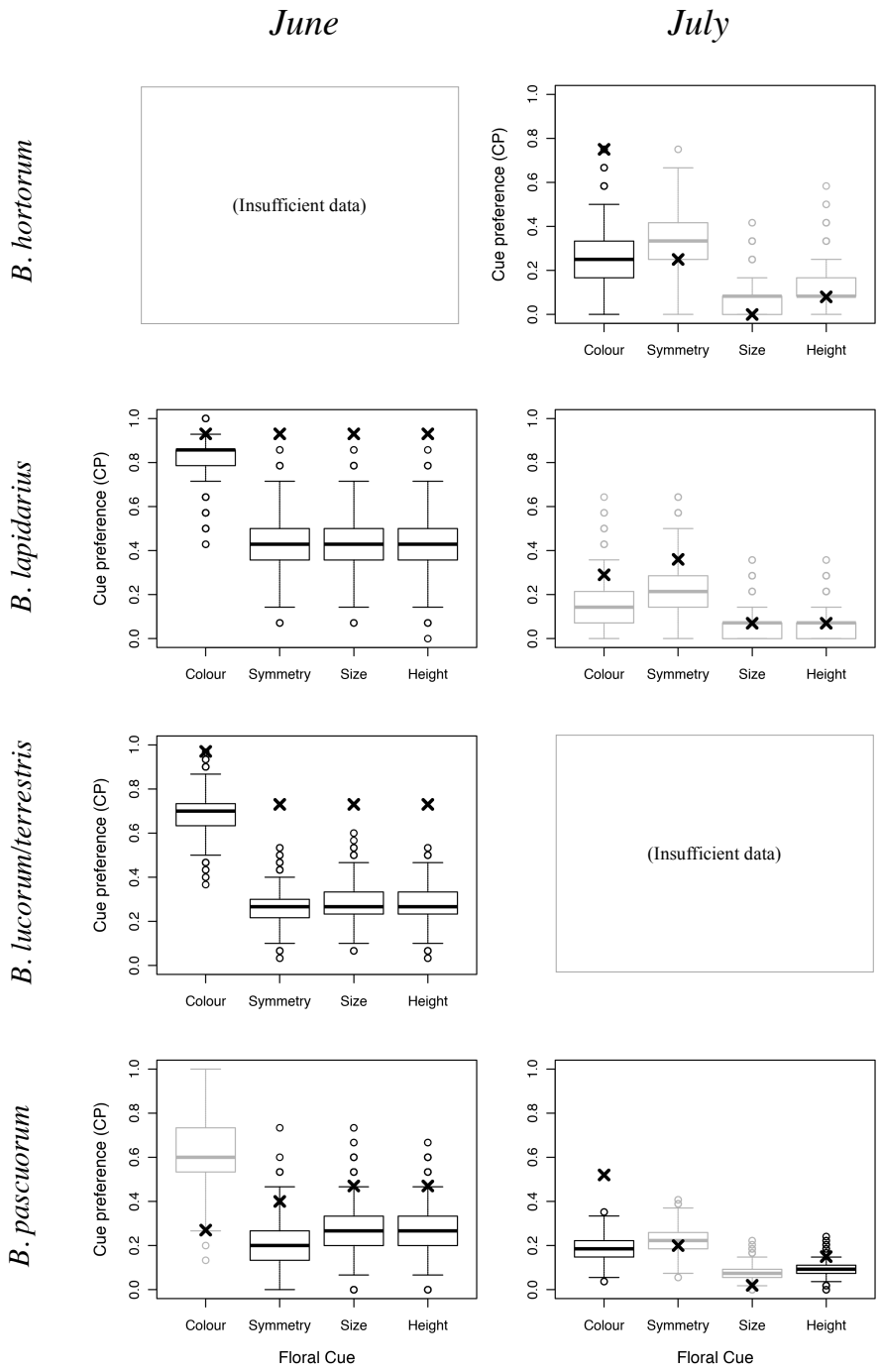


Figure 5.8 The results of the floral units foraging mode for cue preference by month (columns) and for each bumblebee species (rows). Each boxplot shows the distribution of null cue preference values (CP_{null}) and the observed cue preference (CP_{obs}) indicated by the cross. Boxplots for significant cues are black, non-significant cues are plotted in grey.

5.6 Discussion

In order to forage efficiently, Darwin's Interference Hypothesis suggests that bumblebees should develop a preference for high reward plant species *i.e.* individuals should be flower constant. However, bumblebees must also be able to adapt to the dynamic foraging environment (as we saw in Chapter 3 and 4). Therefore, bumblebees must have some mechanism to switch to feeding on different plant species as a new species flower. Bumblebee foraging has been well studied though the precise mechanisms by which bumblebee species chose to forage on different plant species at an individual, colony of species level is complex. Field studies have focused on general aspects of foraging behaviour while (largely lab-based) experiments have looked at isolated aspects of bumblebee foraging preferences and learning. We observed bumblebee individuals in a resource rich meadow and analysed their foraging patterns in terms of constancy and switching behaviour.

We collected focal follow data for four common UK species (*Bombus hortorum, Bombus lapidarius, Bombus lucorum/terrestris* and *Bombus pascuorum*), and found that in all but one, the majority of individuals were constant, visiting one plant species for 5 sequential visits. In all cases, the level of constancy was much higher than would be expected if bumblebee individuals were foraging randomly on the available resource. These results are in line with our expectation that bumblebees will be constant in order to forage efficiently. A study by Heinrich (1979c) found that *Bombus vagans* individuals developed constancy (or 'majoring' as he calls it) on a highly rewarding plant species after as few as 3 flower visits. Interestingly we found that the proportion of constant bumblebee individuals was different between the four bumblebee species and different between June and July when the floral resource was changed. This suggests that the number of constant individuals is species specific and is not fixed, it can change through time as bumblebees adapt to the turnover of plant species in their foraging environment. Future work on understanding constancy in bumblebees should consider differences between bumblebee species and changes over seasonal timescales.

We found our focal follow method to be particularly successful and were able to record a richer data set than expected. Using this data, we were able to quantify constancy in the four bumblebee species (*Bombus hortorum, Bombus lapidarius, Bombus lucorum/terrestris* and *Bombus pascuorum*) using our constancy weighted foraging model. This model takes the immediately available resource of each bumblebee individual (the patch data) into account. To the best of our knowledge, this is the first study to quantify constancy. In contrast to the fact that the proportion of constant individuals was different in

June compared to July, we found that the constancy (*c*) of individuals did not differ between June and July. This suggests that the constancy of individuals does not significantly change throughout the season, but we see changes in the number of constant individuals due to the changing foraging environment. In order to explore this further, we suggest quantifying constancy at throughout a flowering season to measure how constancy changes over time. We found that constancy did not differ when using floral units or nectar provision in the model. This is most likely due to the association between highly abundant and high reward plant species and suggests either measure can be used.

We found that *Bombus pascuorum* individuals that were constant were significantly larger in body length than individuals that switched. It is possible that this is a result of a correlation between task allocation and morphology. However, evidence for strict task allocation is weaker for bumblebees (Jandt, Huang and Dornhaus, 2009). Shpigler et al., (2013) found that body size of worker bumblebees was positively correlated with colony age; bumblebees born early in the colony cycle had a smaller adult body length than individuals born later in the colony cycle. Therefore, it is possible that our observed difference in size between switchers and non-switchers is linked to when in the colony life cycle bumblebee individuals were born.

Despite the high level of constancy, the fraction of bumblebee individuals observed switching was not insubstantial. From the patch data for bumblebees that switched, we found evidence to suggest that bumblebee species had significant preferences for some floral cues. Many studies (mainly laboratory based) have shown that bumblebees are able to recognise, have innate preferences for, and can learn associations with various floral cues (Lunau, Wacht and Chittka, 1996; Goulson *et al.*, 1998; Goulson, 1999; Blarer, Keasar and Shmida, 2002; Raguso, 2002; Lunau, 2016). We believe our study is the first study to explore preference for multiple floral cues in a wild community of bumblebee species at a resource rich site. However, our analysis was limited by our simple qualitative assessment of floral cues. In addition, floral cues are likely to be associated and it is possible that bumblebees can process combinations of cues. For example, we found that *B. lapidarius* showed significant preferences in colour, symmetry, size and height in June. Most *B. lapidarius* visits were to *Rhinathus minor* (yellow rattle) and *Lotus corniculatus* (birdsfoot trefoil). These two species are the same in terms of the floral cues we analysed which may be why we see a signal for all four cues. In addition, there may be confounding effects from other floral cues that we were not able to include in our analysis such as scent (Raguso, 2002) and electric fields (Clarke *et al.*, 2013). Cue preference was not consistent between June and July, suggesting that cue preference changes over time with the foraging environment. Further study is

required to understand preferences for multiple cues in combination and how these change over time.

It is possible that preferences and constancy expressed at a species level might be the result of communication between bumblebee individuals, as is well understood in honeybees. On the other hand, it may be a result of shared experience that differs between species due to biological or environmental factors. Individual bumblebees may have their own foraging preferences and flower fidelity based on individual experience, though we were not able to test for difference between individuals. We suspect that there must be some level of colony level communication that allows individuals to forage more efficiently and assists with the turnover of floral resources. In future work we would like to examine the foraging preferences as we have in the study but taking into account colony level effects by determining which individuals are from the same colony, and individual preferences by giving bumblebees unique markings and collecting data for the same individuals over time.

Overall, we have shown that in a resource rich meadow, the majority of bumblebee individuals observed are flower constant for 5 consecutive flower visits and that individuals that switch have significant preferences that can be detected at the species level. We developed a new model that can quantify constancy (c). We have shown that although c varies between bumblebee species, it is similar even when the floral resource has changed from June to July. This opens up the opportunity for future studies to compare constancy across other bumblebee species and examine the relationship between constancy and factor such as colony lifespan, worker turn over, resource availability and different habitats. Our results suggest that it is possible to explore constancy to floral cues in wild populations. Compared to laboratory-based experiments of bumblebee foraging behaviour, observed foraging in the wild is complicated by external factors that cannot be controlled. However, studies in wild populations are vital to inform the overall picture and help piece together evidence from more focused laboratory experiments. We also suggest that this is an area of research that could be explored further by quantifying cues and accounting for the overlapping effects of multiple cues. Crucially, understanding the foraging behaviour of bumblebee species and individuals, as well as other insect pollinators, will help inform our understanding of how plant-pollinator communities change over time which will be a major step forward in our ability to protect them.

Chapter 6

Conclusions

6.1 Overview of our findings and future directions

Global declines in a broad range of pollinator species and threats to plant-pollinator communities are well documented (Gill *et al.,* 2016; Hallmann *et al.,* 2017). These declines are concerning not just in terms of the loss of biodiversity and the disruption of food webs but also because of the key role that pollinators play in providing ecosystem services that are vital to humans (Losey & Vaughan, 2012). We advocate a networks approach to explore and understand plant-pollinator communities as it enables us to examine phenomena across many species and account for community dynamics.

Recent pollinator network research has focused on theoretical approaches and modelling techniques. In Chapter 2, we explored network robustness models that can be used to model extinctions in plant-pollinator networks, as well as other types of ecological network. We developed a new robustness model and a method to compare the outcomes of different models (based on those developed by Memmott, Waser and Price, 2004; Kaiser-Bunbury *et al.*, 2010; Pocock, Evans and Memmott, 2012; Vieira and Almeida-Neto, 2015). This development ultimately allowed us to determine how the calculated robustness was affected by model mechanism and the network data used. We found that the robustness was affected by model choice, with some models producing opposing effects and therefore over- or underestimating robustness. All of the robustness models we examined produced a wide range of robustness results, a fact that is often overlooked. We found that the breadth of this range is driven by structural heterogeneity of the network, specifically by a skewed degree distribution with a small number of highly connected species. This structural feature is common in plant-pollinator network data due to the ecology of the community; there tends to be a few highly abundant generalists in a community (Bascompte *et al.,* 2007). However, species which are highly abundant and well-connected change through the season and year on year due to ecological progression. Taking the example of the Ashton Court network used in Chapter 2, *Daucus carota* (wild carrot) is highly abundant and well connected which means it has larger impact on robustness and could be interpreted as being a key species in the robustness of the plant-pollinator community. However, if the Ashton Court network data had been collected a month earlier, it is unlikely that *D. carota* would be so abundant and connected; some other species would hold that position as shown in Chapter 3. The key point here is that the timescale over which the network data is collected, and how it is aggregated is important. A snapshot network is only representative of the community in that short time period, and conversely a network that is aggregated over many survey periods is not representative of the network at a given point in time as demonstrated by CaraDonna *et*

al. (2017). Therefore, we decided to explore how plant-pollinator communities change over time, specifically in terms of how much variation there is and how pollinator foraging behaviour plays a role in a changing community.

In order to better understand the level of changes that a plant pollinator community undergoes we collected plant and bumblebee phenological data over two consecutive flowering seasons (in 2016 and 2017). In Chapter 3 we presented this phenological data and showed how the floral resource changed throughout the season, with different plant species reaching peak flowering at different times. As we expected, some plant species flowered throughout the season whereas others only flowered for a short period. This meant that the foraging resource available to pollinators at any given time fluctuated and changed throughout the season. From our bumblebee observations we determined that the common bumblebee species at the site (5 species) had colony lifespans that outlived many of the flowering plant species. Therefore, bumblebee species have to adapt to changes in their foraging environment in order to survive, at least at the colony level and possibly at an individual level. To quantify this, we measured turnover in the bumblebee-plant interactions networks we recorded and found that turnover was high. There are three key types of turnover that can occur in a plant-pollinator community over time and that will be realised as a difference in the networks sampled at different times; 1) plant species flowering turnover, 2) pollinator turnover and 3) interaction turnover. Overall, we determined that the structure of our observed plant-pollinator community (in a temperate environment with a fairly long flowering season) changes as plant species flower at different times and pollinator species change in both their phenology and adapt to the changing foraging resources. We agree with CaraDonna *et al.* (2017) that 'future studies of fine-scale temporal dynamics of interactions and their drivers…will greatly improve our ability to predict the reshuffling of communities in the face of ongoing change'.

In order to test interaction reshuffling in the field, we designed a pulse perturbation experiment that measured the effect of the removal of an abundant plant species (*C. eriophorum*) on the plant-pollinator community. We found that the network structure significantly changed as a result of the perturbation but healed once *C. eriophorum* had re-flowered. In addition, we showed that knock-on effects spread out through the network to other plant and pollinator species. This indicates that the loss of a plant species can impact the community as a whole, not just the pollinators that interact with the plant species directly. These results strongly support the use of networks as a valuable approach to understanding the impacts of species loss or else impacts on species not directly affected may be missed. Despite being able to model the magnitude of effect on species based on

their position in the network, we were not able to predict the effect of the loss of *C. eriophorum* on plants and pollinators in terms of degree and interactions, most likely because of the diversity of effects and the range of abundant and rare species. The degree and number of observed interactions of some species increased whilst others decreased as a result of the perturbation. Future work could focus on understanding the variety of responses to the loss of the key plant species in the community, in the same way that we were able to show the variety of responses in three bumblebee species. We found that the three abundant bumblebee species that were the primary visitors to *C. eriophorum* had different responses; *B. lapidarius* switched to feeding on other plant species that it previously hadn't been observed on, *B. lucorum/terrestris* left the site (presumably to forage elsewhere) and *B. pascuorum* fed on the plant species it had been visiting before. All three species returned to feeding mostly on *C. eriophorum* once it had re-flowered. We initially expected that the three bumblebee species would react in a similar manner to the loss of *C. eriophorum* so the fact that their responses were so clearly different was particularly interesting to us. The loss of *B. lucorum/terrestris* from the site fits well with the assumptions made in the simple knock-on extinction models in Chapter 2 (Dunne, Williams and Martinez, 2002; Memmott, Waser and Price, 2004; Pocock, Evans and Memmott, 2012) that the loss of a plant species leads to the loss of connected pollinator species. However, the response of *B. lapidarius* and *B. pasucoum* contradict this modelling assumption and would be better modelled with some form of re-wiring such as that employed by Kaiser-Bunbury *et al.* (2010). However, it seems that a greater understanding of how pollinator species rewire is needed, as *B. lapidarius* and *B. pascuorum* rewired differently. A key message from Chapter 4 was that the plant-pollinator network responded quickly to the removal and regrowth if a key plant species, indicating that the network has plasticity and that pollinator species are able to change their behaviour in order to adapt to a sudden change in their foraging environment. This led us to focus on the foraging behaviour of individual bumblebees in Chapter 5 in order to understand how bumblebees might be able to adapt to changes in their environment whilst maintaining efficient foraging.

In Chapter 5, we presented focal follow observations, used to collect data on the foraging patterns of four bumblebee species; *B. hortorum, B. lapidarius, B. lucorum/terrestris* and *B. pascuorum.* We found that, in agreement with the theory of foraging economics (*e.g.* Goulson, 1999; Davies, Krebs and West, 2012) and Darwin's interference hypothesis (see Freeman, 1968), bumblebee individuals tended to exhibit flower constancy. We developed a new method to quantify constancy using a foraging expectation model that accounts for the forage resources in the immediate vicinity of an individual

bumblebee. We found that the four bumblebee species had different foraging preferences and the levels of constancy were significantly different between species, in agreement with our observations of foraging bumblebees in Chapter 4. However, we were particularly interested in the bumblebee individuals that did not exhibit flower constancy. Very few of these individuals were switching between plant species in line with a random foraging expectation based on plant abundance. This suggests that there was some other factor driving switching behaviour. We explored 4 floral traits and found some evidence to suggest that these could be linked to the observed switching; in some cases, bumblebee species were significantly constant to one or more floral traits. However, we were only able to use coarse, qualitative measures of floral traits, and only looked at 4 traits. We suggest that future work could look at floral traits in more detail, and in combination (as many plant species share floral traits and bumblebees may be able to process several floral traits at once).

Overall, this thesis presents an exploration of plant-pollinator networks from both the modelling and field experiment perspective. We found that though modelling tools are undoubtedly valuable, good plant-pollinator network data is vital and that understanding the temporal dynamics of plant-pollinator communities over a range of timescales is key. It is not enough to take published plant-pollinator network data and analyse them without consideration of the timescale that they represent. A plant pollinator network is snapshot of a dynamic community, and a static moment in a highly dynamic system. In contrast to the data used in them, many of the robustness models we explored considered robustness over an undefined scale of ecological time. We present evidence of dynamic variation over sequentially shorter timescales, from fluctuations in species present in a community over a period of two years, to rewiring of a network over a period of a month and finally switching behaviour of individual bumblebees within the timescale of 5 consecutive flowering visits. This evidence overwhelmingly supports the concerns voiced by us and many others about our understanding and correct application of plant-pollinator network data. We suggest further exploration of plant-pollinator interactions and the parallel development of network modelling techniques in order to improve the growing field of ecological networks and help protect pollination services.

References

Alarcón, R., Waser, N. M. and Ollerton, J. (2008) 'Year-to-year variation in the topology of a plant-pollinator interaction network', *Oikos*, 117(12), pp. 1796–1807. doi: 10.1111/j.0030-1299.2008.16987.x.

Albrecht, M., Riesen, M. and Schmid, B. (2010) 'Plant-pollinator network assembly along the chronosequence of a glacier foreland', *Oikos*, 119(10), pp. 1610–1624. doi: 10.1111/j.1600-0706.2010.18376.x.

Alford, D. V. (1969) 'A study of the hibernation of bumblebees (Hymenoptera:Bombidae) in Southern England', *The Journal of Animal Ecology*, 38(1), p. 149. doi: 10.2307/2743.

Almeida-Neto, M., Guimarães, P., Guimarães, P. R., Loyola, R. D. and Ulrich, W. (2008) 'A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement', *Oikos*, 117(8), pp. 1227–1239. doi: 10.1111/j.0030-1299.2008.16644.x.

Amano, T., Smithers, R. J., Sparks, T. H. and Sutherland, W. J. (2010) 'A 250-year index of first flowering dates and its response to temperature changes.', *Proceedings of the Royal Society B: Biological Sciences*. The Royal Society, 277(1693), pp. 2451–7. doi: 10.1098/rspb.2010.0291.

Bailes, E. J., Ollerton, J., Pattrick, J. G. and Glover, B. J. (2015) 'How can an understanding of plant–pollinator interactions contribute to global food security?', *Current Opinion in Plant Biology*, 26, pp. 72–79. doi: 10.1016/j.pbi.2015.06.002.

Balfour, N. J., Ollerton, J., Castellanos, M. C. and Ratnieks, F. L. W. (2018) 'British phenological records indicate high diversity and extinction rates among late-summer-flying pollinators', *Biological Conservation*, 222, pp. 278–283. doi: 10.1016/j.biocon.2018.04.028.

Ballantyne, G., Baldock, K. C. R. and Willmer, P. G. (2015) 'Constructing more informative plant–pollinator networks: visitation and pollen deposition networks in a heathland plant community', *Proceedings of the Royal Society B: Biological Sciences*, 282(1814), p. 20151130. doi: 10.1098/rspb.2015.1130.

Banašek-Richter, C., Cattin, M.-F. and Bersier, L.-F. (2004) 'Sampling effects and the robustness of quantitative and qualitative food-web descriptors', *Journal of Theoretical Biology*, 226(1), pp. 23–32. doi: 10.1016/S0022-5193(03)00305-9.

Bane, M. S., Pocock, M. J. O. and James, R. (2018) 'Effects of model choice, network structure, and interaction strengths on knockout extinction models of ecological robustness', *Ecology and Evolution*, 8(22), pp. 10794–10804. doi: 10.1002/ece3.4529.

Bartko, J. J. (1966) 'The Intraclass Correlation Coefficient as a measure of reliability', *Psychological Reports*, 19(1), pp. 3–11. doi: 10.2466/pr0.1966.19.1.3.

Bartomeus, I., Gravel, D., Tylianakis, J. M., Aizen, M. A., Dickie, I. A. and Bernard-Verdier, M. (2016) 'A common framework for identifying linkage rules across different types of interactions', *Functional Ecology*, 30(12), pp. 1894–1903. doi: 10.1111/1365-2435.12666.

Bartomeus, I., Vilà, M. and Santamaría, L. (2008) 'Contrasting effects of invasive plants in plant-pollinator networks', *Oecologia*. Springer-Verlag, 155(4), pp. 761–770. doi: 10.1007/s00442-007-0946-1.

Barwell, L. J., Isaac, N. J. B. and Kunin, W. E. (2015) 'Measuring β - diversity with species abundance data', *Journal of Animal Ecology*. Edited by T. Coulson, 84(4), pp. 1112–1122.

doi: 10.1111/1365-2656.12362.

Bascompte, J., Jordano, P., Melian, C. J. and Olesen, J. M. (2003) 'The nested assembly of plant-animal mutualistic networks', *Proceedings of the National Academy of Sciences*, 100(16), pp. 9383–9387. doi: 10.1073/pnas.1633576100.

Bastolla, U., Fortuna, M. A., Pascual-García, A., Ferrera, A., Luque, B. and Bascompte, J. (2009) 'The architecture of mutualistic networks minimizes competition and increases biodiversity', *Nature*, 458(7241), pp. 1018–1020. doi: 10.1038/nature07950.

Baude, M., Kunin, W. E., Boatman, N. D., Conyers, S., Davies, N., Gillespie, M. A. K. K., Morton, R. D., Smart, S. M. and Memmott, J. (2016) 'Historical nectar assessment reveals the fall and rise of floral resources in Britain', *Nature*, 530(7588), pp. 85–88. doi: 10.1038/nature16532.

Bawa, K. S. (1990) 'Plant-pollinator interactions in tropical rain forests', *Annual Review of Ecology and Systematics*, 21(1), pp. 399–422. doi: 10.1146/annurev.es.21.110190.002151.

Bellard, C., Thuiller, W., Leroy, B., Genovesi, P., Bakkenes, M. and Courchamp, F. (2013) 'Will climate change promote future invasions?', *Global Change Biology*, 19(12), pp. 3740–3748. doi: 10.1111/gcb.12344.

Benton, T. (2006) *Bumblebees, The natural history and identification of the species found in Britain*. London: HarperCollins.

Bertsch, A. (1984) 'Foraging in male bumblebees (*Bombus lucorum L.*): maximizing energy or minimizing water load?', *Oecologia*, 62(3), pp. 325–336. doi: 10.1007/BF00384264.

Biella, P., Ollerton, J., Barcella, M. and Assini, S. (2017) 'Network analysis of phenological units to detect important species in plant-pollinator assemblages: can it inform conservation strategies?', *Community Ecology*, 18(1), pp. 1–10. doi: 10.1556/168.2017.18.1.1.

Biella, P., Ollerton, J., Tarrant, S. P., Ollerton, J., Tarrant, S., Jersáková, J. and Klečka, J. (2018) 'Experimental loss of generalist plants reveals alterations in plant-pollinator interactions and a constrained flexibility of foraging', *bioRxiv*. doi: 10.1101/279430.

Biesmeijer, J. C. (2006) 'Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands', *Science*, 313(5785), pp. 351–354. doi: 10.1126/science.1127863.

Blarer, A., Keasar, T. and Shmida, A. (2002) 'Possible mechanisms for the formation of flower size preferences by foraging bumblebees', *Ethology*, 108(4), pp. 341–351. doi: 10.1046/j.1439-0310.2002.00778.x.

Blüthgen, N. (2010) 'Why network analysis is often disconnected from community ecology: A critique and an ecologist's guide', *Basic and Applied Ecology*, 11(3), pp. 185–195. doi: 10.1016/j.baae.2010.01.001.

Blüthgen, Nico, Menzel, F. and Blüthgen, Nils (2006) 'Measuring specialization in species interaction networks', *BMC Ecology*, 6(1), p. 9. doi: https://doi.org/10.1186/1472-6785-6-9.

Borgatti, S. P., Mehra, A., Brass, D. J. and Labianca, G. (2009) 'Network analysis in the social sciences', *Science*, 323(5916), pp. 892–895. doi: 10.1126/science.1165821.

Brosi, B. J., Niezgoda, K. and Briggs, H. M. (2017) 'Experimental species removals impact the architecture of pollination networks', *Biology Letters*, 13(6), p. 20170243. doi: 10.1098/rsbl.2017.0243.

Burgos, E., Ceva, H., Perazzo, R. P. J., Devoto, M., Medan, D., Zimmermann, M. and María Delbue, A. (2007) 'Why nestedness in mutualistic networks?', *Journal of Theoretical Biology*, 249(2), pp. 307–313. doi: 10.1016/j.jtbi.2007.07.030.

Burkle, L. A., Marlin, J. C. and Knight, T. M. (2013) 'Plant-pollinator interactions over 120 years: Loss of species, co-occurrence, and function', *Science*. American Association for the Advancement of Science, 340(6127), pp. 1611–1615. doi: 10.1126/science.1232728.

Campbell, C., Yang, S., Albert, R., Shea, K. and Kadanoff, L. P. (2010) 'A network model for plant–pollinator community assembly', *PNAS*, 108(1), pp. 197–202. doi: 10.1073/pnas.1008204108.

Campbell, C., Yang, S., Shea, K. and Albert, R. (2012) 'Topology of plant-pollinator networks that are vulnerable to collapse from species extinction', 86(2), p. 021924. doi: 10.1103/PhysRevE.86.021924.

CaraDonna, P. J., Petry, W. K., Brennan, R. M., Cunningham, J. L., Bronstein, J. L., Waser, N. M. and Sanders, N. J. (2017) 'Interaction rewiring and the rapid turnover of plant–pollinator networks', *Ecology Letters*, 20(3), pp. 385–394. doi: 10.1111/ele.12740.

Carstensen, D. W., Sabatino, M., Trøjelsgaard, K. and Morellato, L. P. C. (2014) 'Beta diversity of plant-pollinator networks and the spatial turnover of pairwise interactions', *PLoS ONE*, 9(11), p. e112903. doi: 10.1371/journal.pone.0112903.

Carvalheiro, L. G., Biesmeijer, J. C., Benadi, G., Fründ, J., Stang, M., Bartomeus, I., Kaiser-Bunbury, C. N., Baude, M., Gomes, S. I. F., Merckx, V., Baldock, K. C. R., Bennett, A. T. D., Boada, R., Bommarco, R., Cartar, R., Chacoff, N., Dänhardt, J., Dicks, L. V, Dormann, C. F., *et al.* (2014) 'The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness.', *Ecology letters*, 17(11), pp. 1389–99. doi: 10.1111/ele.12342.

Carvell, C., Roy, D. B., Smart, S. M., Pywell, R. F., Preston, C. D. and Goulson, D. (2006) 'Declines in forage availability for bumblebees at a national scale', *Biological Conservation*, 132(4), pp. 481–489. doi: 10.1016/j.biocon.2006.05.008.

Chagnon, P.-L. (2015) 'Characterizing topology of ecological networks along gradients: The limits of metrics' standardization', *Ecological Complexity*, 22, pp. 36–39. doi: 10.1016/j.ecocom.2015.01.004.

Chen, I.-C., Hill, J. K., Ohlemuller, R., Roy, D. B. and Thomas, C. D. (2011) 'Rapid range shifts of species associated with high levels of climate warming', *Science*, 333(6045), pp. 1024–1026. doi: 10.1126/science.1206432.

Clarke, D., Whitney, H., Sutton, G. and Robert, D. (2013) 'Detection and learning of floral electric fields by bumblebees', *Science*, 340(6128), pp. 66–69. doi: 10.1126/science.1230883.

Clements, R. E. and Long, F. L. (1923) *Experimental pollination: an outline of the ecology of flowers and insects*. Washington DC: Carnegie Institure of Washington.

Cnaani, J., Thomson, J. D. and Papaj, D. R. (2006) 'Flower choice and learning in foraging bumblebees: Effects of variation in nectar volume and concentration', *Ethology*, 112(3), pp. 278–285. doi: 10.1111/j.1439-0310.2006.01174.x.

Croft, D. P., James, R. and Krause, J. (2008) *Exploring animal social networks*. Princeton University Press.

Culley, T. M., Weller, S. G. and Sakai, A. K. (2002) 'The evolution of wind pollination in angiosperms', *Trends in Ecology & Evolution*, 17(8), pp. 361–369. doi: 10.1016/S0169-5347(02)02540-5.

Czaran, T. and Bartha, S. (1992) 'Spatiotemporal dynamic models of plant populations and communities', *Trends in Ecology & Evolution*, 7(2), pp. 38–42. doi: 10.1016/0169-5347(92)90103-I.

Davies, N. B., Krebs, J. R. and West, S. A. (2012) 'An introduction to behavioural ecology', in. Wiley-Blackwell.

Delmas, E., Besson, M., Brice, M.-H., Burkle, L. A., Dalla Riva, G. V., Fortin, M.-J., Gravel, D., Guimarães, P. R., Hembry, D. H., Newman, E. A., Olesen, J. M., Pires, M. M., Yeakel, J. D. and Poisot, T. (2018) 'Analysing ecological networks of species interactions', *Biological Reviews*. doi: 10.1111/brv.12433.

Devoto, M., Bailey, S., Craze, P. and Memmott, J. (2012) 'Understanding and planning ecological restoration of plant-pollinator networks', *Ecology Letters*, 15(4), pp. 319–328. doi: 10.1111/j.1461-0248.2012.01740.x.

Dicks, L. V., Abrahams, A., Atkinson, J., Biesmeijer, J., Bourn, N., Brown, C., Brown, M. J. F., Carvell, C., Connolly, C., Cresswell, J. E., Croft, P., Darvill, B., De Zylva, P., Effingham, P., Fountain, M., Goggin, A., Harding, D., Harding, T., Hartfield, C., *et al.* (2013) 'Identifying key knowledge needs for evidence-based conservation of wild insect pollinators: a collaborative cross-sectoral exercise', *Insect Conservation and Diversity*, 6(3), pp. 435–446. doi: 10.1111/j.1752-4598.2012.00221.x.

Dicks, L. V., Corbet, S. A. and Pywell, R. F. (2002) 'Compartmentalization in plant-insect flower visitor webs', *Journal of Animal Ecology*. Blackwell Science Ltd, 71(1), pp. 32–43. doi: 10.1046/j.0021-8790.2001.00572.x.

Dicks, L. V., Viana, B., Bommarco, R., Brosi, B., Arizmendi, M. del C., Cunningham, S. A., Galetto, L., Hill, R., Lopes, A. V., Pires, C., Taki, H. and Potts, S. G. (2016) 'Ten policies for pollinators', *Science*, 354(6315), pp. 975–976. doi: 10.1126/science.aai9226.

Dohzono, I. and Yokoyama, J. (2010) 'Impacts of alien bees on native plant-pollinator relationships: A review with special emphasis on plant reproduction', *Applied Entomology and Zoology*, 45(1), pp. 37–47. doi: 10.1303/aez.2010.37.

Dormann, C. F., Frund, J., Bluthgen, N. and Gruber, B. (2009) 'Indices, graphs and null models: Analyzing bipartite ecological networks', *The Open Ecology Journal*, 2(1), pp. 7–24. doi: 10.2174/1874213000902010007.

Dormann, C. F., Gruber, B. and Fruend, J. (2008) 'Introducing the bipartite Package: Analysing Ecological Networks', *R News*, 8(2), pp. 8–11.

Dornhaus, A. and Chittka, L. (2004) 'Information flow and regulation of foraging activity in bumble bees (*Bombus* spp.)', *Apidologie*, 35(2), pp. 183–192. doi: 10.1051/apido:2004002.

Dougherty, M. (1995) 'A review of neural networks applied to transport', *Transportation Research Part C: Emerging Technologies*, 3(4), pp. 247–260. doi: 10.1016/0968-090X(95)00009-8.

Dreisig, H. (1995) 'Ideal free distributions of nectar foraging bumblebees', *Oikos*, 72(2), p. 161. doi: 10.2307/3546218.

Dunne, J. A., Williams, R. J. and Martinez, N. D. (2002) 'Network structure and biodiversity loss in food webs: robustness increases with connectance', *Ecology Letters*, 5(4), pp. 558–567. doi: 10.1046/j.1461-0248.2002.00354.x.

Dupont, Y. L., Trøjelsgaard, K. and Olesen, J. M. (2011) 'Scaling down from species to individuals: a flower-visitation network between individual honeybees and thistle plants', *Oikos*, 120(2), pp. 170–177. doi: 10.1111/j.1600-0706.2010.18699.x.

Elzinga, J. A., Atlan, A., Biere, A., Gigord, L., Weis, A. E. and Bernasconi, G. (2007) 'Time after time: flowering phenology and biotic interactions', *Trends in Ecology & Evolution*, 22(8), pp. 432–439. doi: 10.1016/j.tree.2007.05.006.

Falk, S. (2015) *Field Guide to the Bees of Great Britain and Ireland*. London: British

Wildlife Publishing, Bloomsbury.

Fortuna, M. A. and Bascompte, J. (2006) 'Habitat loss and the structure of plant-animal mutualistic networks', *Ecology Letters*, 9(3). doi: 10.1111/j.1461-0248.2005.00868.x.

Fox, R. (2013) 'The decline of moths in Great Britain: a review of possible causes', *Insect Conservation and Diversity*, 6(1), pp. 5–19. doi: 10.1111/j.1752-4598.2012.00186.x.

Free, J. B. (1955a) 'The collection of food by bumblebees', *Insectes Sociaux*. doi: 10.1007/BF02331508.

Free, J. B. (1955b) 'The division of labour within bumblebee colonies', *Insectes Sociaux*, 2(3), pp. 195–212. doi: 10.1007/BF02224381.

Free, J. B. (1970) *Insect pollination of crops*. London and New York: Academic Press.

Free, J. B. (1970) 'The flower constancy of bumblebees', *The Journal of Animal Ecology*, 39(2), p. 395. doi: 10.2307/2978.

Free, J. B. (1979) 'Bumblebee economics', *Nature*, 280(5725), pp. 850–850. doi: 10.1038/280850a0.

Freeman, R. B. (1968) 'Charles Darwin on the routes of male humble bees', *Bulletin of the British Museum (Natural History)*, 3(6), pp. 177–189.

Frisch, K. von (1950) *Bees: Their Vision, Chemical, Senses and Language*. New York, NY: Cornell University Press. doi: 10.1039/b926219a.

Gallai, N., Salles, J.-M., Settele, J. and Vaissière, B. E. (2009) 'Economic valuation of the vulnerability of world agriculture confronted with pollinator decline', *Ecological Economics*, 68(3), pp. 810–821. doi: 10.1016/j.ecolecon.2008.06.014.

Gamer, M. and Lemon, J. (2012) 'Package "irr"'. Available at: https://cran.r-project.org/web/packages/irr/index.html.

Gibson, R. H., Knott, B., Eberlein, T. and Memmott, J. (2011) 'Sampling method influences the structure of plant-pollinator networks', *Oikos*, 120(6), pp. 822–831. doi: 10.1111/j.1600-0706.2010.18927.x.

Gill, R. J., Baldock, K. C. R., Brown, M. J. F., Cresswell, J. E., Dicks, L. V., Fountain, M. T., Garratt, M. P. D., Gough, L. A., Heard, M. S., Holland, J. M., Ollerton, J., Stone, G. N., Tang, C. Q., Vanbergen, A. J., Vogler, A. P., Woodward, G., Arce, A. N., Boatman, N. D., Brand-Hardy, R., *et al.* (2016) 'Protecting an ecosystem service', in *Advances in Ecological Research*, pp. 135–206. doi: 10.1016/bs.aecr.2015.10.007.

Goldstein, J. and Zych, M. (2016) 'What if we lose a hub? Experimental testing of pollination network resilience to removal of keystone floral resources', *Arthropod-Plant Interactions*, 10(3), pp. 263–271. doi: 10.1007/s11829-016-9431-2.

Goulson, D. (1999) 'Foraging strategies of insects for gathering nectar and pollen, and implications for plant ecology and evolution', *Perspectives in Plant Ecology, Evolution and Systematics*, 2(2), pp. 185–209. doi: 10.1078/1433-8319-00070.

Goulson, D. (2010) *Bumblebees: behaviour, ecology, and conservation*, *Bumblebees: Behaviour, Ecology, and Conservation*. Oxford: Oxford University Press.

Goulson, D., Derwent, L. C. and Peat, J. (2005) 'Evidence for alloethism in stingless bees (Meliponinae)', *Apidologie*, 36(3), pp. 411–412. doi: 10.1051/apido:2005029.

Goulson, D., Lye, G. C. and Darvill, B. (2008) 'Diet breadth, coexistence and rarity in bumblebees', *Biodiversity and Conservation*, 17(13), pp. 3269–3288. doi: 10.1007/s10531-008-9428-y.

Goulson, D., Nicholls, E., Botias, C. and Rotheray, E. L. (2015) 'Bee declines driven by combined stress from parasites, pesticides, and lack of flowers', *Science*, 347(6229), pp. 1255957–1255957. doi: 10.1126/science.1255957.

Goulson, D., Stout, J. C., Hawson, S. A. and Allen, J. A. (1998) 'Floral display size in comfrey, *Symphytum officinale L*. (Boraginaceae): relationships with visitation by three bumblebee species and subsequent seed set', *Oecologia*, 113(4), pp. 502–508. doi: 10.1007/s004420050402.

Goulson, D. and Wright, N. P. (1998) 'Flower constancy in the hoverflies *Episyrphus balteatus* (Degeer) and *Syrphus ribesii* (L.) (Syrphidae)', *Behavioral Ecology*, 9(3), pp. 213–219. doi: 10.1093/beheco/9.3.213.

Gravel, D., Massol, F. and Leibold, M. A. (2016) 'Stability and complexity in model meta-ecosystems', *Nature Communications*, 7(1), p. 12457. doi: 10.1038/ncomms12457.

Grubbs, F. E. (1969) 'Procedures for detecting outlying observations in samples', *Technometrics*, 11(1), pp. 1–21. doi: 10.1080/00401706.1969.10490657.

Gumbert, A. (2000) 'Color choices by bumble bees (*Bombus terrestris*): Innate preferences and generalization after learning', *Behavioral Ecology and Sociobiology*, 48(1), pp. 36–43. doi: 10.1007/s002650000213.

Gursky, S. (2000) 'Effect of seasonality on the behavior of an insectivorous primate, *Tarsius spectrum*', *International Journal of Primatology*, 21(3), pp. 477–495. doi: 10.1023/A:1005444020059.

Hall, S. J. and Raffaelli, D. G. (1993) 'Food Webs: Theory and Reality', in Begon, M. and Fitter, A. H. (eds) *Advances in Ecological Research*. Elsevier, pp. 187–239. doi: 10.1016/S0065-2504(08)60043-4.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D. and de Kroon, H. (2017) 'More than 75 percent decline over 27 years in total flying insect biomass in protected areas', *PLOS ONE*. Edited by E. G. Lamb, 12(10), p. e0185809. doi: 10.1371/journal.pone.0185809.

Hanley, N., Breeze, T. D., Ellis, C. and Goulson, D. (2015) 'Measuring the economic value of pollination services: Principles, evidence and knowledge gaps', *Ecosystem Services*. Elsevier, 14(4), pp. 124–132. doi: 10.1016/j.ecoser.2014.09.013.

Hanson, M. A. and Butler, M. G. (1994) 'Responses to food web manipulation in a shallow waterfowl lake', *Hydrobiologia*, 279–280(1), pp. 457–466. doi: 10.1007/BF00027877.

Hegland, S. J., Nielsen, A., Lázaro, A., Bjerknes, A.-L. and Totland, Ø. (2009) 'How does climate warming affect plant-pollinator interactions?', *Ecology Letters*, 12(2), pp. 184–195. doi: 10.1111/j.1461-0248.2008.01269.x.

Heinrich, B. (1976a) 'Resource partitioning among some eusocial insects: Bumblebees', *Ecology*, 57(5), pp. 874–889. doi: 10.2307/1941054.

Heinrich, B. (1976b) 'The foraging specializations of individual bumblebees', *Ecological Monographs*, 46(2), pp. 105–128. doi: 10.2307/1942246.

Heinrich, B. (1979a) *Bumblebee economics*. Cambridge, Mass.: Harvard University Press.

Heinrich, B. (1979b) '"Majoring" and "Minoring" by foraging bumblebees, *Bombus Vagans*: An experimental analysis', *Ecology*, 60(2), pp. 245–255. doi: 10.2307/1937652.

Heinrich, B. (1979c) 'Resource heterogeneity and patterns of movement in foraging bumblebees', *Oecologia*, 40(3), pp. 235–245. doi: 10.1007/BF00345321.

Heinrich, B. (1983) 'Do bumblebees forage optimally, and does it matter?', *Integrative and*

Comparative Biology, 23(2), pp. 273–281. doi: 10.1093/icb/23.2.273.

Henri, D. C. and Van Veen, F. J. F. (2011) 'Body size, life history and the structure of host–parasitoid networks', in Belgrano, A. (ed.) *Advances in Ecological Research*. Elsevier, pp. 135–180. doi: 10.1016/B978-0-12-386475-8.00004-6.

Herrera, C. M. (1990) 'Daily patterns of pollinator activity, differential pollinating effectiveness, and floral resource availability, in a summer-flowering Mediterranean shrub', *Oikos*, 58(3), p. 277. doi: 10.2307/3545218.

Heuschen, B., Gumbert, A. and Lunau, K. (2005) 'A generalised mimicry system involving angiosperm flower colour, pollen and bumblebees' innate colour preferences', *Plant Systematics and Evolution*, 252(3–4), pp. 121–137. doi: 10.1007/s00606-004-0249-5.

Hickling, R., Roy, D. B., Hill, J. K., Fox, R. and Thomas, C. D. (2006) 'The distributions of a wide range of taxonomic groups are expanding polewards', *Global Change Biology*, 12(3), pp. 450–455. doi: 10.1111/j.1365-2486.2006.01116.x.

Hicks, D. M., Ouvrard, P., Baldock, K. C. R., Baude, M., Goddard, M. A., Kunin, W. E., Mitschunas, N., Memmott, J., Morse, H., Nikolitsi, M., Osgathorpe, L. M., Potts, S. G., Robertson, K. M., Scott, A. V, Sinclair, F., Westbury, D. B. and Stone, G. N. (2016) 'Food for pollinators: Quantifying the nectar and pollen resources of urban flower meadows', *PLOS ONE*, 11(6), p. e0158117. doi: 10.1371/journal.pone.0158117.

Hurlbert, S. H. (1984) 'Pseudoreplication and the design of ecological field experiments', *Ecological Monographs*, 54(2), pp. 187–211. doi: 10.2307/1942661.

Ings, T. C., Montoya, J. M., Bascompte, J., Blüthgen, N., Brown, L., Dormann, C. F., Edwards, F., Figueroa, D., Jacob, U., Jones, J. I., Lauridsen, R. B., Ledger, M. E., Lewis, H. M., Olesen, J. M., van Veen, F. J. F., Warren, P. H. and Woodward, G. (2009) 'Review: Ecological networks - beyond food webs', *Journal of Animal Ecology*, 78(1), pp. 253–269. doi: 10.1111/j.1365-2656.2008.01460.x.

Inoue, M. N. and Yokoyama, J. (2006) 'Morphological variation in relation to flower use in bumblebees', *Entomological Science*, 9(2), pp. 147–159. doi: 10.1111/j.1479-8298.2006.00162.x.

Inouye, D. W. (1980) 'The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblebees', *Oecologia*, 45(2), pp. 197–201. doi: 10.1007/BF00346460.

James, A., Pitchford, J. W. and Plank, M. J. (2012) 'Disentangling nestedness from models of ecological complexity', *Nature*. Nature Publishing Group, 487(7406), pp. 227–230. doi: 10.1038/nature11214.

Jandt, J. M., Huang, E. and Dornhaus, A. (2009) 'Weak specialization of workers inside a bumble bee (*Bombus impatiens*) nest', *Behavioral Ecology and Sociobiology*, 63(12), pp. 1829–1836. doi: 10.1007/s00265-009-0810-x.

Jersáková, J., Johnson, S. D. and Kindlmann, P. (2006) 'Mechanisms and evolution of deceptive pollination in orchids', *Biological Reviews*, 81(02), p. 219. doi: 10.1017/S1464793105006986.

Jordano, P. (2016) 'Sampling networks of ecological interactions', *Functional Ecology*. Edited by D. Stouffer, 30(12), pp. 1883–1893. doi: 10.1111/1365-2435.12763.

Kaiser-Bunbury, C. N., Mougal, J., Whittington, A. E., Valentin, T., Gabriel, R., Olesen, J. M. and Blüthgen, N. (2017) 'Ecosystem restoration strengthens pollination network resilience and function', *Nature*, 542(7640), pp. 223–227. doi: 10.1038/nature21071.

Kaiser-Bunbury, C. N., Muff, S., Memmott, J., Müller, C. B. and Caflisch, A. (2010) 'The

robustness of pollination networks to the loss of species and interactions: A quantitative approach incorporating pollinator behaviour', *Ecology Letters*, 13(4), pp. 442–452. doi: 10.1111/j.1461-0248.2009.01437.x.

Kaiser-Bunbury, C. N., Valentin, T., Mougal, J., Matatiken, D. and Ghazoul, J. (2011) 'The tolerance of island plant-pollinator networks to alien plants', *Journal of Ecology*, 99(1), pp. 202–213. doi: 10.1111/j.1365-2745.2010.01732.x.

Kaiser-Bunbury, C. N., Vázquez, D. P., Stang, M. and Ghazoul, J. (2014) 'Determinants of the microstructure of plant–pollinator networks', *Ecology*. Ecological Society of America, 95(12), pp. 3314–3324. doi: 10.1890/14-0024.1.

Katz, R. W. (2010) 'Statistics of extremes in climate change', *Climatic Change*, 100(1), pp. 71–76. doi: 10.1007/s10584-010-9834-5.

Kearns, C. A., Inouye, D. W. and Waser, N. M. (1998) 'Endangered mutualisms: The conservation of plant-pollinator interactions', *Annual Review of Ecology and Systematics*, 29(1), pp. 83–112. doi: 10.1146/annurev.ecolsys.29.1.83.

Keasar, T., Motro, U., Shur, Y. and Avi, S. (1996) 'Overnight memory retention of foraging skills by bumblebees is imperfect', *Animal Behaviour*, 52(1), pp. 95–104. doi: 10.1006/anbe.1996.0155.

Kelly, A. E. and Goulden, M. L. (2008) 'Rapid shifts in plant distribution with recent climate change', *Proceedings of the National Academy of Sciences*, 105(33), pp. 11823–11826. doi: 10.1073/pnas.0802891105.

Kemp, J. E., Evans, D. M., Augustyn, W. J. and Ellis, A. G. (2017) 'Invariant antagonistic network structure despite high spatial and temporal turnover of interactions', *Ecography*, 40(11), pp. 1315–1324. doi: 10.1111/ecog.02150.

King, M. J. and Buchmann, S. L. (2003) 'Floral sonication by bees: Mesosomal vibration by *Bombus* and *Xylocopa*, but not *Apis* (Hymenoptera: Apidae), ejects pollen from poricidal anthers', *Journal of the Kansas Entomological Society*, 76(762), pp. 295–305. doi: 10.2307/25086116.

Klein, A.-M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C. and Tscharntke, T. (2007) 'Importance of pollinators in changing landscapes for world crops', *Proceedings of the Royal Society B: Biological Sciences*, 274(1608), pp. 303–313. doi: 10.1098/rspb.2006.3721.

Knight, M. E., Martin, A. P., Bishop, S., Osborne, J. L., Hale, R. J., Sanderson, R. A. and Goulson, D. (2005) 'An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species', *Molecular Ecology*, 14(6), pp. 1811–1820. doi: 10.1111/j.1365-294X.2005.02540.x.

Koleff, P., Gaston, K. J. and Lennon, J. J. (2003) 'Measuring beta diversity for presence-absence data', *Journal of Animal Ecology*, 72(3), pp. 367–382. doi: 10.1046/j.1365-2656.2003.00710.x.

Komsta, L. and Novomestky, F. (2015) 'moments'. CRAN. Available at: https://cran.r-project.org/package=moments.

Kraus, F. B., Wolf, S. and Moritz, R. F. A. (2009) 'Male flight distance and population substructure in the bumblebee Bombus terrestris', *Journal of Animal Ecology*, 78(1), pp. 247–252. doi: 10.1111/j.1365-2656.2008.01479.x.

Krebs, C. J. (1999) *Ecological Methodology*. 2nd edn. New York: Addison Wesley Longman.

Laliberté, E. and Tylianakis, J. M. (2010) 'Deforestation homogenizes tropical parasitoid–

host networks', *Ecology*, 91(6), pp. 1740–1747. doi: 10.1890/09-1328.1.

Last, F. (2001) 'Keeping Records', *The Horticulturalist*, 10, pp. 9–11.

Laverty, T. M. (1994) 'Bumble bee learning and flower morphology', *Animal Behaviour*, 47(3), pp. 531–545. doi: 10.1006/anbe.1994.1077.

Laverty, T. M. (1997) 'Costs to foraging bumble bees of switching plant species', *Canadian Journal of Zoology*, 75(4), pp. 659–659. doi: 10.1139/z97-085.

Laverty, T. M. and Plowright, R. C. (1988) 'Flower handling by bumblebees: a comparison of specialists and generalists', *Animal Behaviour*, 36(3), pp. 733–740. doi: 10.1016/S0003-3472(88)80156-8.

Leiss, K. A. and Klinkhamer, P. G. L. (2005) 'Spatial distribution of nectar production in a natural Echium vulgare population: Implications for pollinator behaviour', *Basic and Applied Ecology*, 6(4), pp. 317–324. doi: 10.1016/j.baae.2005.02.006.

Lenoir, J., Gegout, J. C., Marquet, P. A., de Ruffray, P. and Brisse, H. (2008) 'A significant upward shift in plant species optimum elevation during the 20th century', *Science*, 320(5884), pp. 1768–1771. doi: 10.1126/science.1156831.

Lewis, A. C. (1986) 'Memory constraints and flower choice in *Pieris rapae*', *Science*, 232(4752), pp. 863–865. doi: 10.1126/science.232.4752.863.

Lopezaraiza-Mikel, M. E., Hayes, R. B., Whalley, M. R. and Memmott, J. (2007) 'The impact of an alien plant on a native plant-pollinator network: An experimental approach', *Ecology Letters*, 10(7), pp. 539–550. doi: 10.1111/j.1461-0248.2007.01055.x.

Losey, J. E. and Vaughan, M. (2006) 'The economic value of ecological services provided by insects', *BioScience*, 56(4), pp. 311–323. doi: 10.1641/0006-3568(2006)56[311:TEVOES]2.0.CO;2.

Lubbock, J. (1897) *On British wild flowers considered in relation to insects*. London: Macmillan.

De Luca, P. A. and Vallejo-Marín, M. (2013) 'What's the "buzz" about? The ecology and evolutionary significance of buzz-pollination', *Current Opinion in Plant Biology*, 16(4), pp. 429–435. doi: 10.1016/j.pbi.2013.05.002.

Lunau, K. (2016) 'Flower colour: How bumblebees handle colours with perceptually changing hues', *Current Biology*, 26(6), pp. R229–R231. doi: 10.1016/j.cub.2016.02.004.

Lunau, K., Wacht, S. and Chittka, L. (1996) 'Colour choices of naive bumble bees and their implications for colour perception', *Journal of Comparative Physiology A*, 178(4), pp. 477–489. doi: 10.1007/BF00190178.

Mace, G. M. (2008) 'Millennium Ecosystem Assessment', *Encyclopedia of Life Sciences (eLS)*. John Wiley & Sons. doi: 10.1002/9780470015902.a0020478.

Manning, A. (1956) 'Some aspects of the foraging behaviour of Bumblebees', *Behaviour*, 9(1), pp. 164–200. doi: 10.1163/156853956X00291.

Margary, I. D. (2007) 'The Marsham phenological record in Norfolk, 1736-1925, and some others', *Quarterly Journal of the Royal Meteorological Society*, 52(217), pp. 27–54. doi: 10.1002/qj.49705221705.

Martinez, N. D. (1992) 'Constant connectance in community food webs', *The American Naturalist*, 139(6), pp. 1208–1218. doi: 10.1086/285382.

May, R. M. (1973) *Stability and complexity in model ecosystems*. Princeton University Press.

McMahon, D. P., Fürst, M. a, Caspar, J., Theodorou, P., Brown, M. J. F. and Paxton, R. J.

(2015) ‘A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees’, *Journal of Animal Ecology*. Edited by S. Altizer, 84(3), pp. 615–624. doi: 10.1111/1365-2656.12345.

Memmott, J. (1999) ‘The structure of a plant-pollinator food web’, *Ecology Letters*, 2(5), pp. 276–280. doi: 10.1046/j.1461-0248.1999.00087.x.

Memmott, J., Craze, P. G., Waser, N. M. and Price, M. V. (2007) ‘Global warming and the disruption of plant-pollinator interactions’, *Ecology Letters*. Blackwell Publishing Ltd, 10(8), pp. 710–717. doi: 10.1111/j.1461-0248.2007.01061.x.

Memmott, J., Waser, N. M. and Price, M. V (2004) ‘Tolerance of pollination networks to species extinctions’, *Proceedings of the Royal Society B: Biological Sciences*, 271(1557), pp. 2605–2611. doi: 10.1098/rspb.2004.2909.

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kubler, K., Bissolli, P., BRASLAVSKÁ, O., BRIEDE, A., CHMIELEWSKI, F. M., CREPINSEK, Z., CURNEL, Y., DAHL, Å., DEFILA, C., DONNELLY, A., FILELLA, Y., JATCZAK, K., MÅGE, F., *et al.* (2006) ‘European phenological response to climate change matches the warming pattern’, *Global Change Biology*, 12(10), pp. 1969–1976. doi: 10.1111/j.1365-2486.2006.01193.x.

Menzel, R. and Erber, J. (1978) ‘Learning and memory in bees’, *Scientific American*, 239(1), pp. 102–111. doi: 10.1038/scientificamerican0778-102.

Milo, R. (2002) ‘Network motifs: Simple building blocks of complex networks’, *Science*, 298(5594), pp. 824–827. doi: 10.1126/science.298.5594.824.

Morales-Castilla, I., Matias, M. G., Gravel, D. and Araújo, M. B. (2015) ‘Inferring biotic interactions from proxies’, *Trends in Ecology & Evolution*, 30(6), pp. 347–356. doi: 10.1016/j.tree.2015.03.014.

Neumann, P., Frouz, J., Helenius, J., Sarthou, J., Klein, A., Genersch, E., Kovács-Hostyánszki, A., Samu, F., Stout, J., Pennacchio, F., Berendse, F., van den Berg, M., Fries, I., Norton, M., JP, S., AM, K., E, G., A, K.-H., F, S., *et al.* (2015) *Ecosystem services, agriculture and neonicotinoids*, *EASAC policy report 26*. doi: ISBN 978-3-8047-3437-1.

Newman, M. E. J. (2002) ‘Spread of epidemic disease on networks’, *Physical Review E*, 66(1), p. 016128. doi: 10.1103/PhysRevE.66.016128.

Nieh, J. C. (2004) ‘Recruitment communication in stingless bees (Hymenoptera, Apidae, Meliponini)’, *Apidologie*, 35(2), pp. 159–182. doi: 10.1051/apido:2004007.

Novotny, V. (2009) ‘Beta diversity of plant-insect food webs in tropical forests: a conceptual framework’, *Insect Conservation and Diversity*, 2(1), pp. 5–9. doi: 10.1111/j.1752-4598.2008.00035.x.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O’Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E. and Wagner, H. (2017) ‘vegan: Community Ecology Package’. Available at: https://cran.r-project.org/package=vegan.

Olesen, J. M., Eskildsen, L. I. and Venkatasamy, S. (2002) ‘Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists’, *Diversity and Distributions*. Blackwell Science Ltd, 8(3), pp. 181–192. doi: 10.1046/j.1472-4642.2002.00148.x.

Ollerton, J. (2017) ‘Pollinator diversity: Distribution, ecological function, and conservation’, *Annual Review of Ecology, Evolution, and Systematics*, 48(1), pp. 353–376. doi: 10.1146/annurev-ecolsys-110316-022919.

Ollerton, J., Erenler, H., Edwards, M. and Crockett, R. (2014) 'Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes', *Science*, 346(6215), pp. 1360–1362. doi: 10.1126/science.1257259.

Ollerton, J., Winfree, R. and Tarrant, S. (2011) 'How many flowering plants are pollinated by animals?', *Oikos*, 120(3), pp. 321–326. doi: 10.1111/j.1600-0706.2010.18644.x.

Opsahl, T. (2015) 'Package "tnet": Software for analysis of weighted, two-mode, and longitudinal networks'. Available at: https://toreopsahl.com/tnet/.

Ovaskainen, O., Skorokhodova, S., Yakovleva, M., Sukhov, A., Kutenkov, A., Kutenkova, N., Shcherbakov, A., Meyke, E. and Delgado, M. del M. (2013) 'Community-level phenological response to climate change', *Proceedings of the National Academy of Sciences*. National Academy of Sciences, 110(33), pp. 13434–13439. doi: 10.1073/pnas.1305533110.

Papiorek, S., Junker, R. R., Alves-dos-Santos, I., Melo, G. A. R., Amaral-Neto, L. P., Sazima, M., Wolowski, M., Freitas, L. and Lunau, K. (2016) 'Bees, birds and yellow flowers: pollinator-dependent convergent evolution of UV patterns', *Plant Biology*. Edited by A. Dafni, 18(1), pp. 46–55. doi: 10.1111/plb.12322.

Parmesan, C. and Yohe, G. (2003) 'A globally coherent fingerprint of climate change impacts across natural systems', *Nature*, 421(6918), pp. 37–42. doi: 10.1038/nature01286.

Pastor, J. M., Santamaría, S., Méndez, M. and Galeano, J. (2012) 'Effects of topology on robustness in ecological bipartite networks', *Networks and Heterogeneous Media*, 7(3), pp. 429–440. doi: 10.3934/nhm.2012.7.429.

Pleasants, J. M. (1981) 'Bumblebee response to variation in nectar availability', *Ecology*, 62(6), pp. 1648–1661. doi: 10.2307/1941519.

Pocock, M. J. O., Evans, D. M. and Memmott, J. (2012) 'The robustness and restoration of a network of ecological networks', *Science*, 335(6071), pp. 973–977. doi: 10.1126/science.1214915.

Poisot, T., Canard, E., Mouillot, D., Mouquet, N. and Gravel, D. (2012) 'The dissimilarity of species interaction networks', *Ecology Letters*, 15(12), pp. 1353–1361. doi: 10.1111/ele.12002.

Poisot, T. and Gravel, D. (2014) 'When is an ecological network complex? Connectance drives degree distribution and emerging network properties', *PeerJ*, 2, p. e251. doi: 10.7717/peerj.251.

Poisot, T., Stouffer, D. B. and Gravel, D. (2015) 'Beyond species: why ecological interaction networks vary through space and time', *Oikos*, 124(3), pp. 243–251. doi: 10.1111/oik.01719.

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V, Garibaldi, L. A., Hill, R., Settele, J. and Vanbergen, A. J. (2016) 'Safeguarding pollinators and their values to human well-being', *Nature*, 540(7632), pp. 220–229. doi: 10.1038/nature20588.

Proctor, M., Yeo, P. and Lack, A. (1996) *The Natural History of Pollination* . London: HarperCollins. doi: 10.3366/anh.1997.24.2.307.

Prys-Jones, O. E. E. and Corbet, S. A. A. (2011) *Bumblebees*. 3rd edn. Exeter: Pelagic Publishing.

Pyšek, P., Jarošík, V., Chytrý, M., Danihelka, J., Kühn, I., Pergl, J., Tichý, L., Biesmeijer, J. C., Ellis, W. N., Kunin, W. E. and Settele, J. (2011) 'Successful invaders co-opt pollinators of native flora and accumulate insect pollinators with increasing residence time', *Ecological Monographs*, 81(2), pp. 277–293. doi: 10.1890/10-0630.1.

Quick, N. J., Rendell, L. E. and Janik, V. M. (2008) 'A mobile acoustic localization system for the study of free-ranging dolphins during focal follows', *Marine Mammal Science*, 24(4), pp. 979–989. doi: 10.1111/j.1748-7692.2008.00231.x.

R Core Team (2017) 'R: A language and environment for statistical computing'. Vienna, Austria: R Foundation for Statistical Computing. Available at: http://www.r-project.org/ (Accessed: 10 April 2018).

Raguso, R. A. (2002) 'Cognitive ecology of pollination. Animal behavior and floral evolution', *Ethology*, 108(3), pp. 287–288. doi: 10.1046/j.1439-0310.2002.00774.x.

Raine, N. E. and Chittka, L. (2007) 'Flower constancy and memory dynamics in bumblebees (Hymenoptera : Apidae : *Bombus*)', *Entomologia Generalis*, 29(2–4), pp. 179–199. doi: 0171–8177/07/0029-0179.

Ranta, E. and Lundberg, H. (1980) 'Resource partitioning in bumblebees: The significance of differences in proboscis length', *Oikos*, 35(3), p. 298. doi: 10.2307/3544643.

Rathcke, B. and Lacey, E. P. (1985) 'Phenological Patterns of Terrestrial Plants', *Annual Review of Ecology and Systematics*. doi: 10.1146/annurev.es.16.110185.001143.

Ren, D., Labandeira, C. C., Santiago-Blay, J. A., Rasnitsyn, A., Shih, C., Bashkuev, A., Logan, M. A. V, Hotton, C. L. and Dilcher, D. (2009) 'A probable pollination mode before Angiosperms: Eurasian, long-proboscid Scorpionflies', *Science*. American Association for the Advancement of Science, 326(5954), pp. 840–847. doi: 10.1126/science.1178338.

Renner, S. (2006) 'Rewardless flowers in the angiosperms and the role of insect cognition in their evolution', in Waser, N. M. and Ollerton, J. (eds) *Plant–pollinator Interactions: From Specialization To Generalization*. Illinois: The University of Chicago Press, pp. 123–144.

Rodriguez-Girones, M. A. and Santamaria, L. (2006) 'A new algorithm to calculate the nestedness temperature of presence-absence matrices', *Journal of Biogeography*, 33(5), pp. 924–935. doi: 10.1111/j.1365-2699.2006.01444.x.

Rosi-Denadai, C. A., Araújo, P. C. S., Campos, L. A. de O., Cosme, L. and Guedes, R. N. C. (2018) 'Buzz-pollination in Neotropical bees: genus-dependent frequencies and lack of optimal frequency for pollen release', *Insect Science*. doi: 10.1111/1744-7917.12602.

Sánchez-Bayo, F. and Wyckhuys, K. A. G. (2019) 'Worldwide decline of the entomofauna: A review of its drivers', *Biological Conservation*. doi: 10.1016/j.biocon.2019.01.020.

Santamaría, S., Galeano, J., Pastor, J. M. and Méndez, M. (2016) 'Removing interactions, rather than species, casts doubt on the high robustness of pollination networks', *Oikos*. Blackwell Publishing Ltd, 125(4), pp. 526–534. doi: 10.1111/oik.02921.

Schenk, M., Krauss, J. and Holzschuh, A. (2018) 'Desynchronizations in bee–plant interactions cause severe fitness losses in solitary bees', *Journal of Animal Ecology*. Edited by R. Morris, 87(1), pp. 139–149. doi: 10.1111/1365-2656.12694.

Schweiger, O., Settele, J., Kudrna, O., Klotz, S. and Kühn, I. (2008) 'Climate change can cause spatial mismatch of trophically interacting species', *Ecology*, 89(12), pp. 3472–3479. doi: 10.1890/07-1748.1.

Senapathi, D., Carvalheiro, L. G., Biesmeijer, J. C., Dodson, C.-A., Evans, R. L., McKerchar, M., Morton, R. D., Moss, E. D., Roberts, S. P. M., Kunin, W. E. and Potts, S. G. (2015) 'The impact of over 80 years of land cover changes on bee and wasp pollinator communities in England', *Proceedings of the Royal Society B: Biological Sciences*, 282(1806), pp. 20150294–20150294. doi: 10.1098/rspb.2015.0294.

Shpigler, H., Tamarkin, M., Gruber, Y., Poleg, M., Siegel, A. J. and Bloch, G. (2013) 'Social influences on body size and developmental time in the bumblebee Bombus terrestris',

Behavioral Ecology and Sociobiology, 67(10), pp. 1601–1612. doi: 10.1007/s00265-013-1571-0.

Simmons, B. I., Cirtwill, A. R., Baker, N. J., Wauchope, H. S., Dicks, L. V., Stouffer, D. B. and Sutherland, W. J. (2018) 'Motifs in bipartite ecological networks: uncovering indirect interactions', *Oikos*. doi: 10.1111/oik.05670.

Sirohi, M. H., Jackson, J., Edwards, M. and Ollerton, J. (2015) 'Diversity and abundance of solitary and primitively eusocial bees in an urban centre: a case study from Northampton (England)', *Journal of Insect Conservation*. doi: 10.1007/s10841-015-9769-2.

Small, E. (1976) 'Insect Pollinators of the Mer Bleue peat bog of Ottowa', *Canadian Field-Naturalist*, 90(1), pp. 22–28. Available at: http://www.biodiversitylibrary.org/ (Accessed: 11 May 2017).

Solga, M. J., Harmon, J. P. and Ganguli, A. C. (2014) 'Timing is everything: An overview of phenological changes to plants and their pollinators', *Natural Areas Journal*. Natural Areas Association, 34(2), pp. 227–234. doi: 10.3375/043.034.0213.

Sparks, T. H. and Carey, P. D. (1995) 'The responses of species to climate over two centuries: An analysis of the Marsham phenological record, 1736-1947', *The Journal of Ecology*, 83(2), p. 321. doi: 10.2307/2261570.

Staniczenko, P. P. A., Kopp, J. C. and Allesina, S. (2013) 'The ghost of nestedness in ecological networks', *Nature Communications*, 4. doi: 10.1038/ncomms2422.

Stone, L. and Roberts, A. (1990) 'The checkerboard score and species distributions', *Oecologia*, 85(1), pp. 74–79. doi: 10.1007/BF00317345.

Sunley, R. J., Atkinson, C. J. and Jones, H. G. (2006) 'Chill unit models and recent changes in the occurrence of Winter chill and Spring frost in the United Kingdom', *The Journal of Horticultural Science and Biotechnology*, 81(6), pp. 949–958. doi: 10.1080/14620316.2006.11512181.

Sutherland, W. J. (ed.) (2006) *Ecological Census Techniques: A Handbook*. 2nd edn. Cambridge University Press.

Suzuki, K., Dohzono, I. and Hiei, K. (2007) 'Evolution of pollinator generalization in bumblebee-pollinated plants', *Plant Species Biology*, 22(3), pp. 141–159. doi: 10.1111/j.1442-1984.2007.00187.x.

van Swaay, C. A. M., Nowicki, P., Settele, J. and van Strien, A. J. (2008) 'Butterfly monitoring in Europe: methods, applications and perspectives', *Biodiversity and Conservation*, 17(14), pp. 3455–3469. doi: 10.1007/s10531-008-9491-4.

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B. F. N., Ferreira De Siqueira, M., Grainger, A., Hannah, L., Hughes, L., Huntley, B., Van Jaarsveld, A. S., Midgley, G. F., Miles, L., Ortega-Huerta, M. A., Peterson, A. T., Phillips, O. L. and Williams, S. E. (2004) 'Extinction risk from climate change', *Nature*. doi: 10.1038/nature02121.

Thomson, J. D. (1996) 'Trapline foraging by bumblebees: I. Persistence of flight-path geometry', *Behavioral Ecology*, 7(2), pp. 158–164. doi: 10.1093/beheco/7.2.158.

Timóteo, S., Ramos, J. A., Vaughan, I. P. and Memmott, J. (2016) 'High resilience of seed dispersal webs highlighted by the experimental removal of the dominant disperser', *Current Biology*, 26(7), pp. 910–915. doi: 10.1016/j.cub.2016.01.046.

Toda, N. R. T., Song, J. and Nieh, J. C. (2009) 'Bumblebees exhibit the memory spacing effect', *Naturwissenschaften*, 96(10), pp. 1185–1191. doi: 10.1007/s00114-009-0582-1.

Tooke, F. and Battey, N. H. (2010) 'Temperate flowering phenology', *Journal of Experimental Botany*. doi: 10.1093/jxb/erq165.

Traveset, A., Tur, C. and Eguíluz, V. M. (2017) 'Plant survival and keystone pollinator species in stochastic coextinction models: role of intrinsic dependence on animal-pollination', *Scientific Reports*, 7(1), p. 6915. doi: 10.1038/s41598-017-07037-7.

Tsvetkov, N., Samson-Robert, O., Sood, K., Patel, H. S., Malena, D. A., Gajiwala, P. H., Maciukiewicz, P., Fournier, V. and Zayed, A. (2017) 'Chronic exposure to neonicotinoids reduces honey bee health near corn crops', *Science*, 356(6345), pp. 1395–1397. doi: 10.1126/science.aam7470.

Valiente-Banuet, A., Aizen, M. A., Alcántara, J. M., Arroyo, J., Cocucci, A., Galetti, M., García, M. B., García, D., Gómez, J. M., Jordano, P., Medel, R., Navarro, L., Obeso, J. R., Oviedo, R., Ramírez, N., Rey, P. J., Traveset, A., Verdú, M. and Zamora, R. (2015) 'Beyond species loss: the extinction of ecological interactions in a changing world', *Functional Ecology*, 29(3), pp. 299–307. doi: 10.1111/1365-2435.12356.

Vanbergen, A. J. and Initiative, the I. P. (2013) 'Threats to an ecosystem service: pressures on pollinators', *Frontiers in Ecology and the Environment*, 11(5), pp. 251–259. doi: 10.1890/120126.

Vázquez, D. P., Chacoff, N. P. and Cagnolo, L. (2009) 'Evaluating multiple determinants of the structure of plant-animal mutualistic networks', *Ecology*, 90(8), pp. 2039–2046. doi: 10.1890/08-1837.1.

Vermaat, J. E., Dunne, J. A. and Gilbert, A. J. (2009) 'Major dimensions in food-web structure properties', *Ecology*, 90(1), pp. 278–282. doi: 10.1890/07-0978.1.

Vieira, M. C. and Almeida-Neto, M. (2015) 'A simple stochastic model for complex coextinctions in mutualistic networks: robustness decreases with connectance', *Ecology Letters*. Edited by R. Irwin, 18(2), pp. 144–152. doi: 10.1111/ele.12394.

Vilà, M., Espinar, J. L., Hejda, M., Hulme, P. E., Jarošík, V., Maron, J. L., Pergl, J., Schaffner, U., Sun, Y. and Pyšek, P. (2011) 'Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems', *Ecology Letters*, 14(7), pp. 702–708. doi: 10.1111/j.1461-0248.2011.01628.x.

Walther, G.-R., Roques, A., Hulme, P. E., Sykes, M. T., Pyšek, P., Kühn, I., Zobel, M., Bacher, S., Botta-Dukát, Z. and Bugmann, H. (2009) 'Alien species in a warmer world: risks and opportunities', *Trends in Ecology & Evolution*, 24(12), pp. 686–693. doi: 10.1016/j.tree.2009.06.008.

Waser, N. M. (2007) 'Pollination by Animals', in *Encyclopedia of Life Sciences*. Chichester: John Wiley & Sons, Ltd. doi: 10.1002/9780470015902.a0003163.pub2.

Whittaker, R. H. (1960) 'Vegetation of the Siskiyou Mountains, Oregon and California', *Ecological Monographs*, 30(3), pp. 279–338. doi: 10.2307/1943563.

Williams, I. H. (1994) *The dependence of crop production within the European Union on pollination by honey bees*, *Agricultural Zoology Reviews*. United Kingdom.

Winfree, R., Gross, B. J. and Kremen, C. (2011) 'Valuing pollination services to agriculture', *Ecological Economics*. Elsevier B.V., 71(1), pp. 80–88. doi: 10.1016/j.ecolecon.2011.08.001.

Woodward, G. L. and Laverty, T. M. (1992) 'Recall of flower handling skills by bumble bees: a test of Darwin's interference hypothesis', *Animal Behaviour*, 44(6), pp. 1045–1051. doi: 10.1016/S0003-3472(05)80316-1.

Xia, Y., Yu, H., Jansen, R., Seringhaus, M., Baxter, S., Greenbaum, D., Zhao, H. and

Gerstein, M. (2004) ‘Analyzing cellular biochemistry in terms of molecular networks’, *Annual Review of Biochemistry*, 73(1), pp. 1051–1087. doi: 10.1146/annurev.biochem.73.011303.073950.